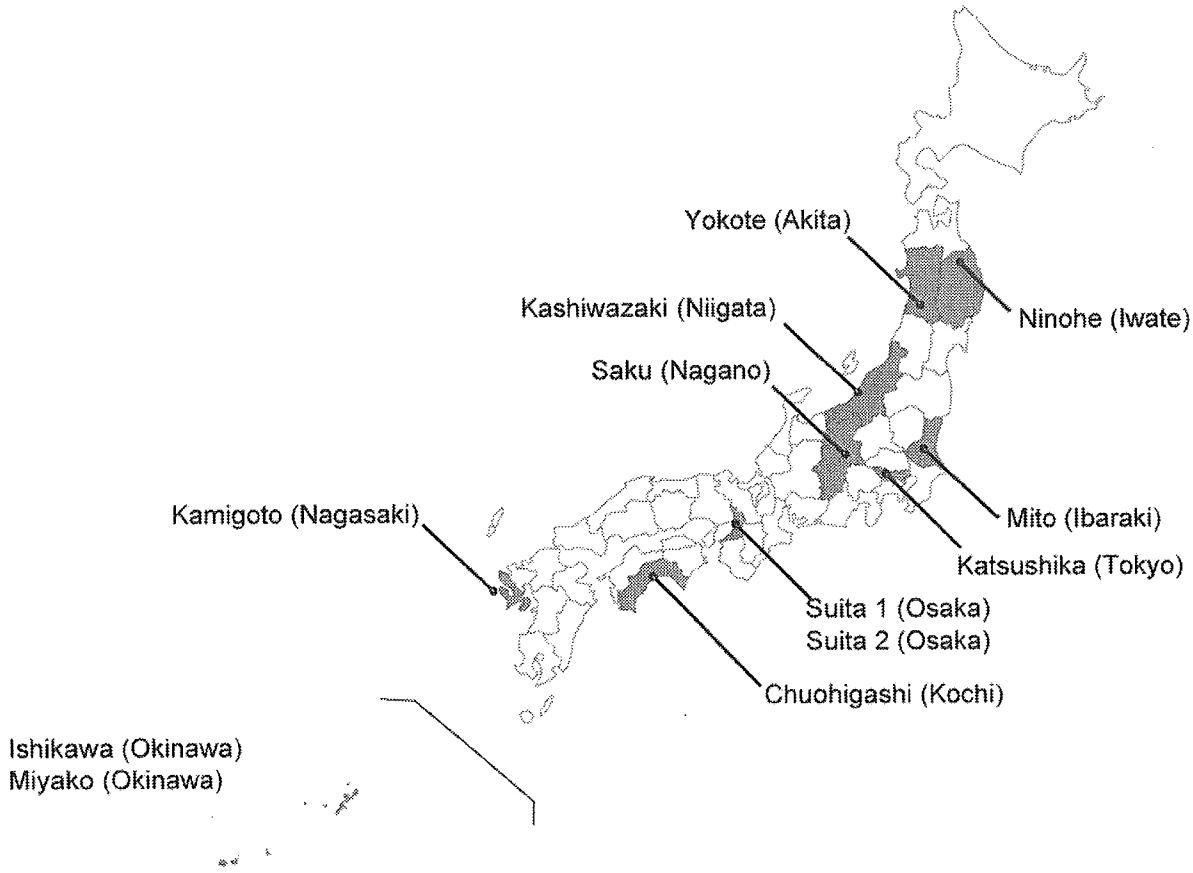

Figure 1 Study sites of the Japan Public Health Center-based prospective study on cancer and cardiovascular disease

characteristics by sex. The odds ratios (OR) with 95% CI were computed using a logistic regression model for the association between dietary supplement use and those factors. *P*-values for linear trends from the lowest to the highest levels of each variable were calculated using logistic regression.

Individual intakes of energy and nutrients were transformed using the natural log scale to normalize skewed distribution. Energy intake was adjusted for area, age group, occupation, smoking, alcohol consumption, physical activity, dietary behaviour, working hours, and stress level using LSMEANS of PROC GLM in SAS. Nutrient intakes were adjusted for the same variables and for energy intake using the same procedure. The geometric means of energy and nutrient intakes by sex were calculated for users and non-users of dietary supplements by the back-transformation of least-square means. The percentage difference was also calculated by dividing the difference in mean intakes between user and non-user by the mean intake of users.

Results

The percentage of dietary supplement users and OR in relation to the reference group by demographics, lifestyles, and health characteristics was shown in Table 1. Among the 12 areas of the JPHC Study, the prevalence was high in urban areas (Katsushika and Suita) and in mainland Okinawa (Ishikawa). Among the 5-year age groups, the prevalence of dietary supplements was lowest in the youngest group, 45–49 years. There was a significant linear increase in dietary supplement users among the higher age groups.

Among the six occupation groups, the self-employed were most likely, and the farming, forestry, and fishing group members were least likely to be supplement users after the adjustment. As for smoking, prevalence of users was significantly lower for current smokers in men, whereas it was significantly higher for former smokers in women. A difference in prevalence by the frequency of alcohol consumption was observed only in women. Women who drank moderately (once a month to 6 times a week) were most likely to be users. For BMI, there was a significant linear decrease of dietary supplement users for higher BMI groups in both sexes. Regarding exercise, there was a significant linear increase in those groups who exercise more frequently.

In the initial analysis of 14 dietary behaviours and the use of dietary supplements, no association was observed with the frequency of eating breakfast, consumption of fried foods, deep-fried foods, and fat on meat, of eating soup from noodle bowls, adding salt or soy sauce to foods at the table, types of vegetable oils used, frequently used cooking methods, well-doneness of cooked meat, and eating charred parts of fish (data not shown). Therefore, we included in the logistic model the only three variables as dietary habits associated with supplement use (frequency of eating miso soup, consumption of prepared foods

Table 1 Percentage of people in the 5-year follow-up survey in Japan Public Health Center-based prospective study on cancer and cardiovascular disease with indicated demographics, lifestyles, and health characteristics of subjects who used dietary supplements

	Male						Female					
	n	User (%)	Odds ratio[a]	95% CI		*P*-value[b]	n	User (%)	Odds ratio[a]	95% CI		*P*-value[b]
Area						–						–
Ninohe	3683	5.6	0.59	0.50	0.70		4362	10.0	0.81	0.72	0.92	
Yokote	5063	10.4	1.09	0.96	1.23		6070	14.5	1.11	1.00	1.23	
Saku	4278	9.4	0.95	0.83	1.08		4390	12.6	0.92	0.82	1.04	
Ishikawa	2996	14.5	1.48	1.29	1.69		3134	24.2	2.12	1.89	2.37	
Katsushika	775	18.6	1.75	1.43	2.15		1178	24.9	1.71	1.46	1.99	
Mito	6792	10.8	1.00[c]				6667	14.7	1.00[c]			
Kashiwazaki	1115	8.5	0.81	0.65	1.02		1105	11.0	0.76	0.62	0.94	
Chuo-higashi	2662	11.1	1.11	0.96	1.29		2921	17.2	1.30	1.15	1.47	
Kamigoto	2979	8.8	0.95	0.81	1.11		3349	13.3	1.03	0.91	1.16	
Miyako	3793	7.4	0.72	0.62	0.84		4215	13.4	1.03	0.92	1.16	
Suita 1	1737	21.9	2.07	1.78	2.41		1954	30.6	2.19	1.92	2.49	
Suita 2	1425	24.0	2.03	1.74	2.37		1888	34.3	2.43	2.15	2.76	
Age (years)						< 0.001						< 0.001
–49	9906	9.1	1.00[c]				10 539	15.0	1.00[c]			
50–54	7151	9.4	1.17	1.05	1.31		7707	15.6	1.24	1.13	1.35	
55–59	8569	13.4	1.52	1.38	1.67		9801	19.0	1.40	1.29	1.51	
60–64	7091	11.3	1.75	1.57	1.96		7807	15.4	1.49	1.36	1.63	
65–69	2667	12.6	2.00	1.71	2.35		3014	17.5	1.71	1.51	1.94	
70–	1914	12.6	2.05	1.71	2.47		2365	17.0	1.73	1.51	1.99	
Occupation												
Farming, forestry, and fishing	6837	6.7	0.66	0.59	0.75		4948	8.6	0.56	0.50	0.63	
Employee or professional	16 225	11.8	1.00[c]				9775	17.2	1.00[c]			
Housewife	–	–	–	–	–		10 594	18.9	0.97	0.89	1.06	
Self-employed	6397	13.0	1.16	1.06	1.27		3814	21.6	1.26	1.14	1.39	
Unemployed	2269	14.1	1.04	0.87	1.24		1695	14.6	0.76	0.65	0.89	
Other occupation	2144	10.4	0.94	0.81	1.10		2299	16.6	0.92	0.81	1.04	
Combination[d]	3426	10.4	1.06	0.94	1.20		8108	14.9	0.88	0.80	0.96	
Smoking						–						–
Current	17 443	9.9	0.87	0.80	0.94		2347	19.4	1.00	0.89	1.12	
Former	6809	13.1	1.06	0.96	1.16		470	28.1	1.40	1.13	1.73	
Never	13 046	11.4	1.00[c]				38 416	16.1	1.00[c]			
Alcohol consumption						0.29						< 0.001
None	8518	10.8	1.00[c]				32 398	15.2	1.00[c]			
1/month–2/week	6299	11.4	1.07	0.96	1.20		5026	20.4	1.26	1.16	1.36	
3–6 times/week	7537	11.8	1.05	0.95	1.16		2350	22.0	1.21	1.08	1.35	
Daily	14 944	10.5	0.97	0.88	1.06		1459	21.3	1.12	0.98	1.28	
Body mass index (kg/m²)						< 0.001						< 0.001
<19	1557	12.1	1.12	0.94	1.33		2512	19.8	1.15	1.03	1.29	
19–<21	5183	11.5	1.06	0.95	1.18		6585	19.5	1.14	1.05	1.24	
21–<23	9145	11.1	1.00[c]				10 923	16.9	1.00[c]			
23–<25	10 785	11.3	1.01	0.92	1.10		9982	16.1	0.96	0.89	1.04	
25–<27	6417	10.7	0.91	0.82	1.01		6187	14.0	0.83	0.76	0.91	
27–<30	3426	9.4	0.80	0.70	0.92		3800	13.3	0.78	0.70	0.87	
≥30	785	8.8	0.75	0.58	0.97		1244	13.7	0.75	0.63	0.89	
Exercise						< 0.001						< 0.001
Never	21 709	9.5	1.00[c]				27 798	14.8	1.00[c]			
1/month–2/week	11713	12.3	1.19	1.11	1.29		9024	19.2	1.22	1.14	1.30	
3 times/week–daily	3876	15.3	1.50	1.35	1.66		4411	21.3	1.38	1.27	1.50	
Miso soup						0.58						0.32
≤2/week	4330	14.0	1.02	0.92	1.14		5508	21.4	1.03	0.95	1.12	
3–6 times/week	9366	12.4	1.03	0.95	1.11		11 426	18.6	1.04	0.98	1.11	
Daily	23 602	9.9	1.00[c]				24 299	14.3	1.00[c]			
Prepared food						< 0.05						< 0.001
Never	10 418	12.0	1.00[c]				17 118	17.0	1.00[c]			
1/month–2/week	24 453	10.6	0.91	0.85	0.98		23 172	16.1	0.91	0.86	0.96	
3 times/week–daily	2427	10.5	0.91	0.78	1.05		943	15.7	0.83	0.69	1.00	
Eating out						< 0.001						< 0.001
Never	11 795	8.1	1.00[c]				16 800	12.3	1.00[c]			
1–3 times/month	12 816	10.9	1.29	1.18	1.41		17 771	18.0	1.42	1.33	1.51	
1–2 times/week	4690	12.6	1.42	1.26	1.59		3960	23.4	1.69	1.54	1.86	
3 times/week–daily	7997	14.6	1.57	1.41	1.74		2702	21.9	1.61	1.44	1.80	

Table 1 continued

	Male						Female					
	n	User (%)	Odds ratio[a]	95% CI		*P*-value[b]	n	User (%)	Odds ratio[a]	95% CI		*P*-value[b]
Working hours						< 0.005						0.60
<5 hours	5099	12.3	1.00[c]				13 724	17.5	1.00[c]			
5–9 hours	24 124	10.3	0.97	0.85	1.11		22 668	15.5	0.93	0.87	0.99	
≥9 hours	8075	12.3	1.13	0.98	1.31		4841	17.8	1.08	0.98	1.19	
Stress level						< 0.001						< 0.001
Low	6891	10.4	1.00[c]				7690	15.3	1.00[c]			
Medium	22 886	10.3	1.06	0.97	1.16		25 941	15.5	1.08	1.00	1.16	
High	7521	13.7	1.42	1.27	1.58		7602	20.6	1.47	1.35	1.61	
Total	37 298	11.0					41 233	16.4				

[a] Variables were mutually adjusted.

[b] *P*-values for linear trend from the lowest to the highest group using logistic regression.

[c] Ref.

[d] Subjects with multiple occupations.

such as freeze-dried noodles and retort-pouched foods, and eating out). For miso soup, although the prevalence of dietary supplement users was highest in the subjects who consumed the least, the difference was not significant after the adjustment for other variables. For prepared foods, the prevalence of users was highest in subjects who never used them. The prevalence of users was highest in the groups with the highest frequency of eating out. There was also a significant linear increase of users in the groups of subjects who eat out more frequently.

Working hours were not associated with dietary supplement use after adjustment for other variables. For stress level, the groups reporting high stress were most likely to be users.

The geometric means of energy and nutrient intakes from diet for users and non-users of any dietary supplement and their percentage difference by sex are shown in Table 2. The mean intake was significantly lower for users as was intake of energy and most nutrients except for sodium and niacin for both sexes, carbohydrate for men, and polyunsaturated fatty acids (PUFA) and selenium for women.

Discussion

In this study, the prevalence of dietary supplement use and its association with demographics, lifestyles, and health characteristics were investigated. The demographics, lifestyle factors, and health characteristics associated with dietary supplement use were sex, age, area of residence, occupation, smoking, BMI, physical activity level, frequency of using prepared foods or eating out, and self-reported stress level. Frequency of alcohol intake was associated only in women. Dietary intake tended to be lower for users of dietary supplements.

A high prevalence of dietary supplement users was observed in metropolitan regions (Suita and Katsushika) and in areas strongly influenced by Western lifestyles (Ishikawa) with ready access to dietary supplements. Associations between supplement use and other demographic factors (sex, age, and occupation) were consistent with results in other studies.[2,4,18–23] In terms of occupation, the lower prevalence in the farming, forestry, and fishing group might reflect their conservative health habits. It might be also a case of one's occupation serving as a surrogate for one's socioeconomic status (SES). A number of studies have indicated a strong association between supplement use and higher income level,[2,4,5,7,20,23] social class,[19] and education.[4,5,20,23,24] Educational background, another factor for SES available only for Cohort I, was also associated with the supplement use.

As for lifestyle and health characteristics, supplement use was associated with healthy lifestyle, which was similar to the earlier-reported tendency for smoking,[4,22,24] BMI, and physical activity.[2,4,7,24,25] Dietary supplement use did not associate with alcohol consumption in men, and was higher in women who drink moderately. Prior studies had reported alcohol consumption in dietary supplement users as either having no association[24] or as showing more users among moderate drinkers.[4,22,26] Supplement users have been characterized as having a positive attitude towards their health. In our study, however, users also showed negative lifestyle factors such as frequent eating out and stressful life. It was assumed that these associations, including moderate drinking among female supplement users, were influenced by urban lifestyle. Such people might be aware of their unhealthy behaviour, and therefore intentionally seek to compensate for it with dietary supplements.

Our results indicated that dietary supplement use could confound the association between dietary intake and disease even after every possible related factor was adjusted. Several earlier studies had found that supplement users consume a more nutrient-dense diet, i.e. low in energy and high in micronutrients.[7,22] In the present study, intakes of both energy and most nutrients were significantly lower for users than non-users of dietary supplements after various factors were adjusted. The results did not change when adjustment was made only for biological factors (age and BMI). The contradiction in the results was assumed to be caused by the complex characteristics of supplement users. Although the SES is usually associated positively with the quality of the diet, some factors such as eating out, which is influenced greatly by SES, can make the association negative.[27] The subgroup analysis in our study indicated that high dietary intake with a higher frequency of eating out made intake of users higher, while low intake with intensive labour such as in farming, forestry, and fishing made intake of users relatively lower.

Table 2 Nutrient intake by supplement users and non-users in the 5-year follow-up survey in Japan Public Health Center Study Cohort II

	Male				Female			
	Users	Non-users			Users	Non-users		
	Mean[c]	Mean[c]	% difference[a]	*P*-value[b]	Mean[c]	Mean[c]	% difference[a]	*P*-value[b]
n	4103	33 195			6776	34 457		
Energy (kcal/day)	1972	2019	−2.4	< 0.01	1774	1829	−3.1	< 0.01
Protein (g/day)	69.8	71.0	−1.8	< 0.01	63.3	64.0	−1.1	< 0.01
Total fat (g/day)	52.0	53.6	−2.9	< 0.01	50.2	51.0	−1.6	< 0.01
Total fatty acid (g/day)	46.4	47.8	−3.0	< 0.01	44.8	45.6	−1.7	< 0.01
SFA[d] (g/day)	15.2	15.9	−4.6	< 0.01	14.7	15.3	−3.9	< 0.05
MUFA[e] (g/day)	19.4	19.9	−2.5	< 0.01	18.7	18.9	−0.9	< 0.05
PUFA[f] (g/day)	11.1	11.4	−2.0	< 0.01	10.8	10.8	−0.5	0.12
n-3 PUFA[f] (g/day)	2.7	2.7	−2.2	< 0.01	2.6	2.6	−0.7	0.12
n-6 PUFA[f] (g/day)	8.4	8.5	−2.0	< 0.01	8.1	8.1	−0.5	0.12
Carbohydrate (g/day)	266.8	266.5	0.1	0.70	228.8	227.5	0.5	< 0.01
Calcium (mg/day)	446	481	−7.8	< 0.01	443	479	−8.3	< 0.01
Phosphorus (mg/day)	1090	1120	−2.7	< 0.01	1000	1028	−2.8	< 0.01
Iron (mg/day)	8.6	8.9	−2.7	< 0.01	8.3	8.4	−1.6	< 0.01
Sodium (mg/day)	3991	4028	−0.9	0.07	3815	3812	0.1	0.83
Potassium (mg/day)	2488	2602	−4.5	< 0.01	2425	2523	−4.0	< 0.01
Retinol (μg/day)	350	362	−3.6	< 0.05	290	309	−6.5	< 0.01
Carotene (μg/day)	1759	1918	−9.0	< 0.01	2023	2148	−6.2	< 0.01
α-carotene (μg/day)	211	235	−11.4	< 0.01	242	261	−7.8	< 0.01
β-carotene (μg/day)	1326	1435	−8.2	< 0.01	1561	1655	−6.0	< 0.01
Lycopene (μg/day)	1173	1417	−20.7	< 0.01	853	1079	−26.5	< 0.01
Vitamin B_1 (mg/day)	1.03	1.05	−2.3	< 0.01	0.95	0.97	−1.8	< 0.01
Vitamin B_2 (mg/day)	1.36	1.44	−5.4	< 0.01	1.32	1.39	−5.5	< 0.01
Niacin (mg/day)	16.7	16.8	−0.8	0.05	15.2	15.2	−0.2	0.60
Vitamin C (mg/day)	101	107	−6.8	< 0.01	107	113	−5.2	< 0.01
Cholesterol (mg/day)	260	268	−3.4	< 0.01	235	239	−1.9	< 0.01
Vitamin B_6 (mg/day)	1.55	1.57	−1.3	< 0.01	1.38	1.40	−1.7	< 0.01
Vitamin B_{12} (μg/day)	8.2	8.4	−2.6	< 0.01	7.4	7.5	−1.5	< 0.05
Folate (μg/day)	251	260	−3.4	< 0.01	243	249	−2.6	< 0.01
Selenium (μg/day)	104	106	−1.6	< 0.01	95	96	−0.7	0.07
Total dietary fibre (g/day)	10.5	11.0	−5.0	< 0.01	10.4	10.9	−4.3	< 0.01
Water-soluble fibre (g/day)	1.5	1.6	−7.8	< 0.01	1.6	1.7	−6.4	< 0.01
Water-insoluble fibre (g/day)	7.0	7.3	−4.2	< 0.01	7.1	7.4	−3.7	< 0.01
Daidzein (mg/day)	8.4	8.9	−5.6	< 0.01	8.0	8.5	−6.4	< 0.01
Genistein (mg/day)	13.8	14.6	−6.0	< 0.01	13.2	14.1	−6.7	< 0.01

[a] % difference was calculated by dividing mean nutrient intake of users minus non-users by mean nutrient intake of users.

[b] *P*-value for difference of geometric mean nutrient intakes between users and non-users.

[c] Geometric mean intake from foods. Energy intake was adjusted for area of residence, age, occupation, smoking, frequency of alcohol consumption, body mass index, physical activity, frequency of miso soup and prepared foods, eating-out, work hours, and stress level. Other nutrients were also adjusted for energy intake in addition to those variables.

[d] Saturated fatty acid.

[e] Monounsaturated fatty acid.

[f] Polyunsaturated fatty acid.

To our knowledge, the present study is the first investigation into the supplement use and its associations with demographics, lifestyles, and health characteristics in a large population in Japan. Only a few smaller studies have so far reported the characteristics of dietary supplement users in Japan.[16,28] The strengths of our study were its population-based large sample, and various and extensive data on potential confounding factors such as demographics, lifestyles, and health characteristics of individuals, as well as dietary supplement use. Those factors can be adjusted later on when the association between supplement use and mortality or disease is investigated. Furthermore, if such association is found, supplement use itself may need to be adjusted when investigating the association between those factors and disease.

One of the limitations of our study was its lack of sensitive SES data, such as income or education level of the participants. Socioeconomic status is strongly associated with supplement use as a result of difference in perception of health and economic status. The positive association with frequency of eating out might be the influence of higher SES level in users, whereas the negative association with prepared food, which tended to be consumed by those who eat at home, might be the influence of low SES.

Generalization of the results could be limited because of the non-respondents to the questionnaire, as well as the representativeness of the study sample. A difference in mortality was observed between the respondents and non-respondents to our baseline questionnaire.[29] For the rural areas where the response rate was 77–90%, the results were probably good estimates for those populations. For the urban areas, however, where the response rate was as low as 40%, supplement use might have been lower in the non-respondents since both the respondents and supplement users tended to have a healthier lifestyle. Furthermore, the overall prevalence might not represent general population in Japan because no statistical weighting

was made for population estimates. However, the characteristics of supplement users were presumably generalizable because the results of subgroup analysis were similar among all areas.

In the present study, we only focused on dichotomous information on dietary supplement use (i.e. user versus non-user) since we aimed to characterize the behaviour of individuals who use dietary supplements. Although we did not examine the amounts consumed or length of dietary supplement use, they may well be of great importance because the association with a disease might depend on them. Further investigation should be done using available data on brand names, frequency, and duration of usage in our study. The development of a database for supplement composition is necessary since it is not currently available.

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KEY MESSAGES

- Dietary supplement users had a healthier and more urban lifestyle than non-users in the Japan Public Health Center-based prospective study on cancer and cardiovascular disease (JPHC Study).
- Dietary supplement use was associated with sex, age, area of residence, smoking, body mass index, physical activity level, frequency of eating prepared food and eating-out, self-reported stress level, and dietary intake. Frequency of alcohol consumption was associated only in women.
- The demographics, lifestyles, health characteristics, and dietary intakes might be adjusted when evaluating the effect of dietary supplements on disease since they can become potential confounding factors. The use of supplements may also become a confounding factor when investigating the association between disease and demographics, lifestyles, health characteristics, or dietary intakes.

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Commentary: Vitamin supplement use and confounding by lifestyle

Katherine J Hoggatt

The role of micronutrients in the development of chronic disease remains unclear. A number of observational studies have suggested a protective effect of various nutrients—e.g. folic acid and vitamin E with coronary heart disease, antioxidant vitamins, and cancer. However, recent reviews of the literature have described inconsistencies among studies and conflict between the results of observational research and clinical trials.[1,2] One explanation for these discrepant findings is that results from observational studies of micronutrient intake and disease may be confounded by variables associated with a 'healthy lifestyle'.[3–6] As one review of supplement use and cancer put it, '[s]upplement use may be a behavioral marker for other factors related to cancer risk ... Control in analyses for

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major health-related behaviors may reduce this confounding effect, but completeness of adjustment can never be assured'.[1] The argument for confounding by lifestyle seems plausible—even in studies of total micronutrient intake, those with the highest intakes tend to be supplement users and numerous studies have shown that supplement use is associated with leading a healthier lifestyle. For example, research in Western populations has found that compared with non-users, supplement users tend to be older and leaner, have higher incomes and education, and are more likely to be female and Caucasian; in addition, they are less likely to smoke or drink heavily and generally consume healthier diets and exercise more than non-users do.[7–12]

Several recent studies have described supplement use in new populations and have expanded our understanding of supplement users (e.g. suggesting that supplement users tend to believe in a diet—cancer connection, are more likely to receive cancer-screening tests, take other medications for disease prevention, and have different underlying medical conditions than do non-users[1,9,10,13]). The current article by Ishihara *et al.*[14] is an interesting addition to the literature—it is one of the few conducted in an Asian population,[15,16] and it reports associations for some factors that have not been widely studied, including several that are specific to a Japanese population. Of particular interest are the results suggesting that while supplement use in Japan tends to be associated with aspects of a Western lifestyle, the '... intakes of both energy and most nutrients were ... lower for users than non-users of dietary supplements after various factors were adjusted'. This contrasts with results in Western populations that suggest supplement users consume healthier diets than do non-users.[8,11]

Adjusting for confounding by lifestyle is complicated, even with the information provided by studies such as the current one. For example, several studies suggest that those who take individual-vitamin supplements differ from those who take multivitamins or do not use supplements at all, and there may be differences between those who take one individual supplement versus another.[9,17,18] Many researchers collect detailed data on supplement use as part of a comprehensive nutrition assessment. For example, the authors of the current study asked about '... general use of any vitamin supplements more than once a week, and use of specific supplements by five categories ... For each category, the brand names, frequency, and duration of use were asked.' The authors also tried to ensure correct classification by '[re-categorizing supplements] using brand names according to the definition of dietary supplements in the Women's Healthy Living Eating and Living Study', and they validated this method in another study.[19] Yet in their analyses, the authors considered only a binary 'user versus non-user' outcome, with users defined as anyone '... who used at least one category of dietary supplement one or more times a week for a year or more'. Results for 'supplement users' can obscure important differences, and when a comparison between high and low intake may be a comparison of those who take individual-vitamin supplements versus those who do not,[20] this heterogeneity is important.

A factor related to supplement use (however 'use' is defined) may or may not be a confounder depending on what other variables are controlled in the model. The authors addressed this concern in their dietary-intake analyses (Table 2), stating '... the results [for the full multivariate models] did not change when adjustment was made only for biological factors (age and BMI)'. Their other results (Table 1) are derived from a model in which the variables are mutually adjusted (although the authors do not explain what this means, I assumed the results were from a multivariate model with all variables entered simultaneously). The authors do not say whether these findings would have changed upon using a different subset of the variables in the model. In describing their model selection, they state that they looked at 14 dietary behaviours in relation to supplement use, and '... included in the logistic model the only three variables as dietary habits associated with supplement use'. But they do not say how they decided what constituted an association. Their text hints that this decision was made by significance tests. In particular their Results section describes associations primarily as 'statistically significant' or not (presumably at the 0.05 level, although this is not stated). However, statistical significance says little about the magnitude or precision of an estimated association, and is especially misleading when its absence is misinterpreted as absence of an association. Absence of significance signifies only that the association was estimated too imprecisely to determine the direction with confidence, and often reflects more the limited size of the sample than the size of the association. This and numerous other problems with significance tests have led many methodologists and editors to actively discourage their use in favour of CI and related techniques.[21–29] Table 1 presents CI for many associations, but the Results section suggests that the authors interpreted (and perhaps disregarded) certain associations based more on their 'statistical significance' than their magnitude or precision.

When a model selection strategy uses statistical significance as a criterion for including or excluding variables, it can lead to downwardly biased estimates for the coefficient standard errors (ref. 29, p. 402). In Table 1 we are only shown results from one final model, with little description of how the model was chosen or the sensitivity of the results to its specification; hence, in the present context we cannot know the severity of this bias. Nonetheless, based on methodological studies cited elsewhere (ref. 29, p. 402), I suspect that the results reported by Ishihara *et al.* are much less accurate (and less significant!) than their CI and *P*-values convey.

Finally, the authors give little detail on how their variables were measured, and they do not say whether the cutpoints used for the lifestyle variables reflect the categories used on the questionnaire or if they were chosen by some other criterion. For example, it is not clear how to use their finding that supplement use is associated with stress, when no detail is given on how they determined an individual's stress level or what the categories 'High', 'Medium', and 'Low' represent. For other variables, the cutpoints reflect understandable quantities, but it is still not clear how they were chosen. (The authors do not say if these details are given in other papers based on these data, but they do give references for in-press articles that may have this information.[30,31]) Poor category choices can obscure dose—response relations and leave unnecessary residual confounding (ref. 29, pp. 205–07). At a minimum, it would be helpful to know if their results were sensitive to their choice of categories.

Of course, the issues raised above apply to other studies of supplement use: many have employed a dichotomous supplement-use variable, and their results may have been

sensitive to how variables were measured, categorized, and modelled. The current study is valuable in providing results in a seldom-studied population of supplement users. As the authors point out, '[f]urther investigation should be done using available data on brand names, frequency, and duration of usage in our study'. Given the wealth of data the authors have collected, I will look forward to seeing future results.

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The effect of 5-year vitamin C supplementation on serum pepsinogen level and *Helicobacter pylori* infection

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We conducted a population-based, double-blind, randomized controlled trial to examine the effect of vitamin C supplementation on serum pepsinogen (PG) level, *Helicobacter pylori* (*H. pylori*) infection, and cytotoxin-associated gene A (*Cag A*) status. Subjects aged 40 to 69 years living in one village in Akita prefecture, a high-risk area for gastric cancer in Japan, were recruited through annual health check-up programs. Among 635 subjects diagnosed as having chronic gastritis on the basis of serum PG levels, after excluding ineligible cases, 439 subjects were assigned to one of four groups using a 2×2 factorial design (0 or 15 mg/day β-carotene and 50 or 500 mg/day vitamin C). However, based on the results from two β-carotene trials in the United States, we discontinued β-carotene (vitamin C supplementation was continued). Finally, 120 subjects in the low-dose group (vitamin C 50 mg), and 124 subjects in the high-dose group (vitamin C 500 mg) completed the 5-year supplementation. The difference in the change of PGI/II ratio between baseline and after 5-year follow up was statistically significant between the intervention groups among those who completed the supplementation: −0.25 for the low-dose group and −0.13 for the high-dose group (*P*=0.046). To conclude, vitamin C supplementation may protect against progression of gastric mucosal atrophy. (Cancer Sci 2003; 94: 378–382)

The association between low intake of vegetables and fruits and epithelial, non-hormone-dependent cancers is one of the most consistent findings in epidemiologic studies.[1] Among many compounds contained in vegetables and fruits, much attention has been focused on antioxidants, including vitamin C, as they are especially abundant in these foods.[1] Ascorbic acid is known to exert a preventative effect against gastric carcinogenesis through its ability to inactivate oxygen free-radicals, as well as to inhibit nitrosoamine formation.[2,3] Based on the above-mentioned findings and biological plausibility, vitamin C seems to have potential as a chemopreventive agent in interventional trials.

A possible relation between *Helicobacter pylori* (*H. pylori*) infection and ascorbic acid is also under investigation, as some authors have suggested that high-dose vitamin C may inhibit *H. pylori* infection.[4,5]

Previous studies have shown that serum pepsinogen (PG) levels reflect the morphological and functional status of the gastric mucosa and may thus be useful for the screening of gastric atrophy, which is a precancerous lesion of gastric cancer.[6] Herein we describe a population-based, double-blind, randomized controlled trial to examine the effects of vitamin C supplementation on serum PG level as a marker of atrophic gastritis and *H. pylori* infection.

Materials and Methods

Our methods have been described in detail[7] and are briefly summarized below.

Participants. Subjects aged 40 to 69 years living in four municipalities (three towns and one village) of Yokote Public Health Center District in Akita prefecture, one of the regions with the highest mortality from gastric cancer in Japan, were recruited through annual health check-up programs for circulatory diseases conducted by each municipality under the National Health and Welfare Services Law for the Aged. Eligibility required diagnosis with chronic atrophic gastritis (defined as PGI<70 ng/ml and PGI/PGII ratio<3.0), no past history of gastric cancer, gastric surgery, liver cancer, cirrhosis, or other cancers within the last 5 years, no abnormal liver function (aspartate aminotransferase>100 IU/liter, alanine aminotransferase>100 IU/liter, or alkaline phosphatase>800 IU/liter), no use of diet supplements containing β-carotene or vitamin C, and no expectation of moving outside the study area within 1 year. Written informed consent was obtained from each participant and the Ethics Committee of the National Cancer Center approved the protocol.

Study design and procedures. At first we conducted a "run-in phase," offering full doses of β-carotene (15 mg/day) and vitamin C (500 mg/day) to all participants for 4 weeks to identify and exclude at an early stage the subjects who either did not comply or showed side effects. Remaining participants were then randomized to one of four groups for 5-year supplementation using a 2×2 factorial design (0 or 15 mg/day β-carotene and 50 or 500 mg/day vitamin C).

Participants were asked to visit community centers every 3 months, where public health nurses checked clinical symptoms and compliance (by counting numbers of unconsumed capsules), and provided further capsules. Health condition (including lipid profile, liver function tests) was also monitored every year at the annual health check-up for circulatory diseases. Blood samples were drawn and stored three times (at baseline, and after the first, and the fifth year) in order to measure serum level of ascorbic acid and *H. pylori* antibody and twice (at baseline and the fifth year) for serum levels of PGs, and cytotoxin-associated gene A (*Cag A*) antibody. The participants were also asked to complete a semiquantitative food frequency questionnaire at baseline and the fifth year inquiring about smoking habits, alcohol consumption, and medical history, as well as dietary habits.

However, in response to a National Cancer Institute press re-

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port released on January 18, 1996, indicating that two β-carotene trials had shown no benefit and potential harm,[8] we were obliged to amend the study protocol. First, β-carotene was stopped, but prescription of vitamin C was continued for 5 years. Second, the study area was restricted to the village where participants had already been recruited, and no new participants were recruited from the three other municipalities. Finally, the primary endpoint of the trial was changed from the 10-year cumulative incidence of gastric cancer to the 5-year change in serum levels of PGs and other biomarkers. We explained in detail the results of the two US studies and the amendment of the study protocol, and collected the discontinued capsules from each participant. Signed consent was obtained again from individuals willing to remain in the study, and new capsules containing vitamin C only (50 mg/day or 500 mg/day) were provided. The vitamin C dosage of 50 mg was set based on current Recommended Dietary Allowance (RDA).[9] According to a recent report,[10] safe doses of vitamin C are less than 1000 mg daily, while bioavailability declines and the absorbed amount is largely excreted at single doses of 500 mg and higher. Thus, we set 500 mg as the dosage for the high-dose group.

Laboratory analysis. Fasting blood samples collected at baseline and after 5 years were analyzed for serum ascorbic acid levels, PGI, PGII, *H.pylori* antibody, and Cag A status. The subjects were asked not to eat or drink anything except water after 9 PM on the day before blood sampling. The serum was sampled be-

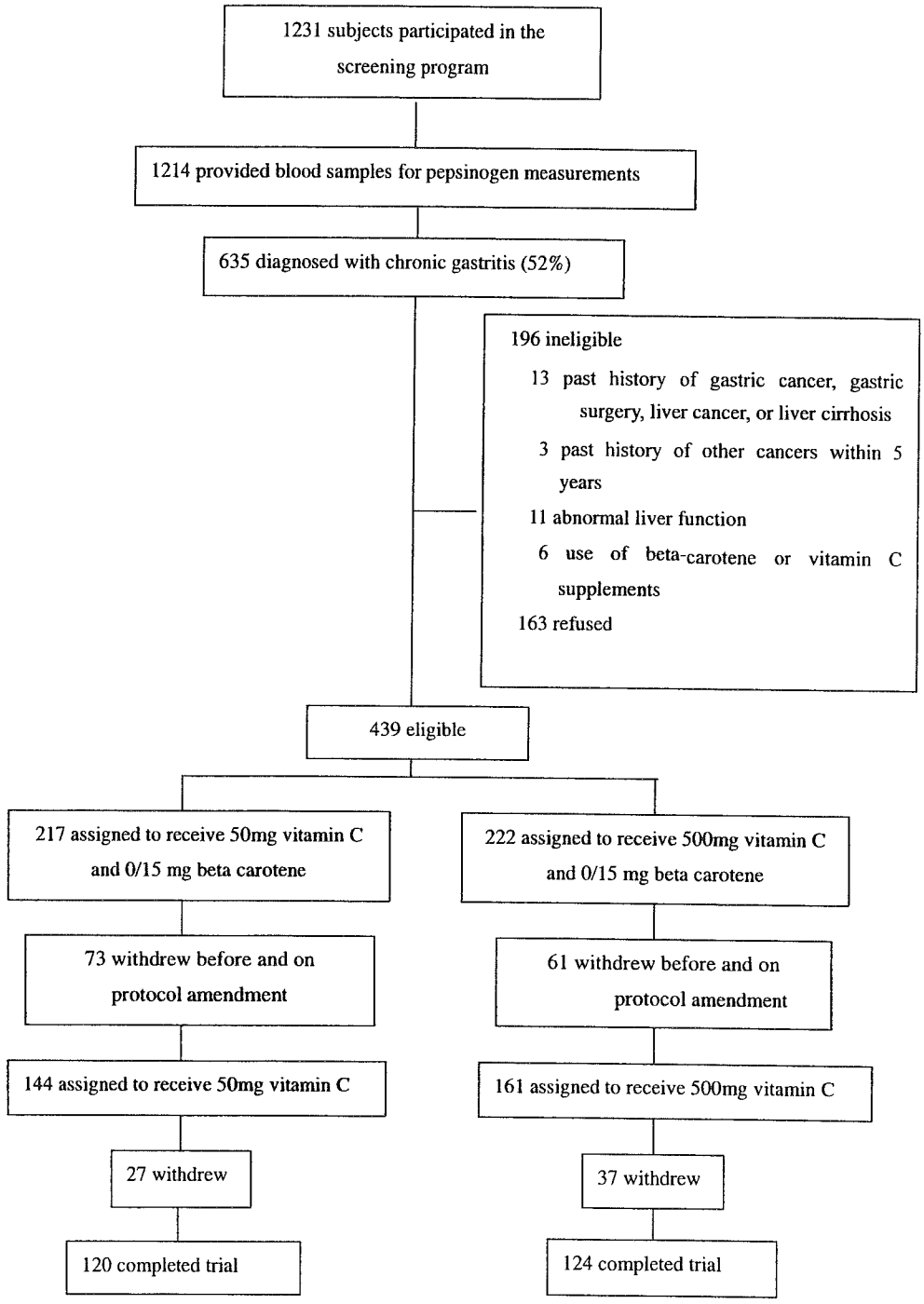

Fig. 1. Study flow.

tween 7 and 10 AM. All samples were stored at −70 to −85°C and were analyzed simultaneously after the completion of the 5-year supplementation. All assays were conducted by persons who were blinded as to the intervention assignment and the questionnaire data.

Serum for ascorbic acid measurement was stabilized by addition of *meta*-phosphoric acid. The level of serum ascorbic acid was analyzed fluorimetrically (iodine oxidation and condensation with 1,2-phenylenediamine). Serum levels of PGI and PGII were measured by radioimmunoassay (RIA) in a commercial laboratory (DINABOT, SRL Co., Ltd., Tokyo). Assays of Cag A was performed by enzyme-linked immunosorbent assay (ELISA), with horseradish peroxidase as the enzyme tracer (Cag A immunoglobulin G (IgG) EIA, Sceti Co., Ltd., Tokyo). The anti-Cag A IgG antibody concentration in standards and samples was measured in a spectrophotometer at 450 nm. IgG antibodies to *H. pylori* were measured with a direct ELISA kit (E Plate 'Eiken' *H. pylori* Antibody, Eiken Kagaku Co., Ltd., Tokyo). Levels of IgG were categorized as seropositive and seronegative for *H. pylori* according to the selected cut off value (492 nm).

Statistical analysis. Baseline characteristics by randomized group were assessed using one-way analysis of variance (ANOVA) for continuous variables and Fisher's exact test for discrete variables. The *P* value was calculated for the difference between the low-dose group and high-dose group. The paired *t* test or one-sample *Z* test for rates was used for analysis of within-group change of several biomarkers or seropositive rates. One-way ANOVA or one-sample *Z* test for rates were conducted to investigate the difference of change in serum biomarkers between groups. Multivariate ANOVA and multiple logistic analysis was conducted to control the effect of sex, age and baseline level of *H.pylori* titer. As the distribution of *H. pylori* titer was skewed to the right side, natural logarithms of *H. pylori* titer were always used in the statistical analysis. Reported *P* values were two-sided, and all statistical analyses were done by using the Statistical Analysis System (SAS) version 6.12 (SAS Institute, Inc., Cary, NC).

Results

A total of 1231 subjects, aged 40 to 69 years, entered the annual health check-up program for circulatory diseases which was conducted from June through July, 1995 (Fig. 1). Among them, 1214 subjects provided blood samples for PG measurements and 635 (52%) were diagnosed as having chronic gastritis on the basis of PG level. One hundred and ninety-six men were considered unfit for further examination because they did not meet the inclusion criteria: 13 had a past history of gastric cancer, gastric surgery, liver cancer, or liver cirrhosis; 3 had a past history of other cancers within 5 years; 11 had abnormal liver function; 6 reported use of β-carotene or vitamin C supplements; 163 refused to participate. Finally, 439 subjects (73%) participated in the study. After amendment of the protocol, 305 men (144 men assigned for taking 50 mg of vitamin C and 161 men assigned for taking 500 mg of vitamin C) remained in the study (intention-to-treat cohort). During the follow up, 24 subjects of the 50 mg assigned group and 37 subjects of the 500 mg assigned group withdrew from the study. Thus, 120 subjects for the 50 mg group and 124 subjects for the 500 mg group completed the supplementation (completed group). Subjects for analysis were restricted to those who had supplied blood both at baseline and after 5 years of follow up. This left 117 subjects for each intervention group. Results of completed group analysis are presented in the tables. The same analyses were repeated for the intention-to-treat group, and essentially similar results were obtained.

Baseline characteristics were compared between the low-dose group (vitamin C supplemented with 50 mg), the high-dose group (vitamin C supplemented with 500 mg) and the drop-out group (drop-out before modification of the protocol) (Table 1). No statistically significant difference was found, including the baseline level of serum ascorbic acid, between the intervention groups. Baseline levels of titer of *H. pylori* and Cag A and PG levels were also compared between the intervention groups. No difference was seen, except in the log-transformed *H. pylori* titer; 4.05 for low-dose group and 4.33 for high-dose group ($P=0.005$).

Table 2 shows the changes between baseline and after 5 years of follow up of serum ascorbic acid, *H. pylori* infection, Cag A, and PG levels within the vitamin C supplemented groups and the difference in the changes between groups (completed group analysis). Serum ascorbic acid significantly increased in both groups and the change was significantly higher in the high-dose group ($P=0.0001$). For *H. pylori* and Cag A status, more subjects became seronegative in the high-dose group, though the difference in percentage was significant only for Cag A. As for PG status, about 10% of subjects were not determined as having atrophic gastritis serologically in both groups. While statistically significantly enhanced values of PGII and a decreased

Table 1. Baseline characteristics by randomized group

	Intervention group			
	Low dose (vitamin C 50 mg) *n*=117	High dose (vitamin C 500 mg) *n*=117	*P* for difference[1)]	Drop out[2)] *n*=134
Age	59.3 (0.6)	57.4 (0.7)	0.05	57.8 (0.7)
Male (%)	33.3	35.0	0.89	35.8
Current smoker (%)	10.3	13.7	0.55	13.4
Alcohol drinker, 1+/week (%)	36.8	40.2	0.69	24.6
Body mass index (kg/m^2)	23.4 (0.3)	23.3 (0.3)	0.72	24.0 (0.3)
Dietary intakes				
Vitamin C (mg/day)	150.9 (9.7)	153.4 (9.6)	0.86	133.8 (8.5)
Fruit (g/day)	205.7 (20.3)	211.2 (20.5)	0.85	157.9 (12.5)
Green or yellow vegetables (g/day)	56.3 (5.3)	56.4 (5.4)	0.98	50.9 (4.2)
Other vegetables (g/day)	140.6 (13.8)	151.6 (13.9)	0.58	128.6 (12.1)
Serum ascorbic acid (mg/dl)	1.38 (0.03)	1.35 (0.03)	0.49	1.4 (0.03)

Values are means (SE) unless otherwise specified.
1) Difference between low-dose group and high-dose group. Based on one-way analysis of variance (ANOVA), or Fisher's exact test.
2) Drop out before design modification.

Table 2. Change in serum ascorbic acid, *Helicobacter pylori (H. pylori)*, Cag A, and pepsinogen (PG) level between baseline (i) and after 5-year follow up (ii) and difference of the changes (ii–i) between randomized groups —completed group analysis

	Within-group change						Between group difference		
	Low dose (vitamin C 50 mg) (n=117)			High dose (vitamin C 500 mg) (n=117)			Low dose ii–i	High dose ii–i	P for difference[2]
	1995 (i)	2000 (ii)	P[1]	1995 (i)	2000 (ii)	P[1]			
Ascorbic acid (mg/dl)	1.38 (1.32–1.44)	1.48 (1.42–1.54)	0.002	1.35 (1.29–1.41)	1.73 (1.65–1.81)	0.0001	0.10 (0.02–0.18)	0.37 (0.29–0.45)	0.0001
H. pylori status,[3] no. (%)	113 (96.6%)	111 (94.9%)	0.40	116 (99.1%)	113 (96.6%)	0.14	−1.7%	−2.6%	0.47
ln *H. pylori* titer (U/ml)	4.05 (3.91–4.19)	3.97 (3.81–4.13)	0.04	4.33 (4.32–4.34)	4.24 (4.08–4.40)	0.04	−0.07 (−0.17–0.03)	−0.09 (−0.19–0.01)	0.75
Cag A status,[3] no. (%)	98 (83.8%)	100 (85.5%)	0.61	106 (90.6%)	105 (89.7%)	0.75	1.7%	−0.9%	<0.001
Cag A titer (RU/ml)	101.20 (87.24–115.16)	89.72 (76.34–103.1)	0.02	118.81 (104.49–133.13)	108.70 (93.97–123.40)	0.05	−11.48 (−21.34–−1.62)	−10.11 (−19.97–−0.25)	0.85
PG status,[3] no. (%)	117 (100%)	105 (89.7%)	<0.001	117 (100%)	106 (90.6%)	<0.001	−10.3%	−9.4%	0.74
PGI (ng/ml)	37.91 (34.78–41.04)	39.09 (37.14–42.95)	0.24	39.58 (36.57–42.59)	41.22 (37.60–44.84)	0.15	1.18 (−0.78–3.14)	1.65 (−0.45–3.75)	0.76
PGII (ng/ml)	19.28 (17.97–20.59)	22.78 (20.92–24.64)	0.0001	20.93 (19.56–22.30)	24.10 (22.28–25.92)	0.0001	3.50 (2.33–4.67)	3.17 (2.00–4.34)	0.70
PGI/II ratio	1.97 (1.85–2.09)	1.71 (1.65–1.83)	0.0001	1.89 (1.79–1.99)	1.76 (1.64–1.88)	0.01	−0.25 (−0.33–−0.17)	−0.13 (−0.21–−0.05)	0.05

Values are means (95% CI) unless otherwise specified.
1) Based on paired t test or one-sample Z test for rates.
2) Based on one-way analysis of variance (ANOVA) or one-sample Z test for rates.
3) Number and percentage of subjects positive for *H. pylori* and Cag A, and serologically determined atrophic gastritis for PG (PGI<70 ng/ml and PGI/PGII ratio<3.0).

ratio of PGI/II between baseline and after 5 years of follow up were observed for both groups, the change between the two groups was only significant for PGI/II ratio; −0.25 in the low-dose group, and −0.13 in the high-dose group (P for difference=0.046). Although the strength of the relation was slightly diminished, the overall result did not differ essentially when the same analysis was conducted on the intention-to-treat basis (P=0.06).

Adjusted mean concentrations of change in serum ascorbic acid, titer of *H. pylori* and Cag A, and PGs between baseline and after 5-year follow up were compared between the intervention groups. After adjustment for sex and age, and also baseline level of *H. pylori* titer, the difference in change of serum ascorbic acid and PGI/II ratio between baseline and 5-year follow up between intervention groups still remained statistically significant (P=0.02) (completed group analysis). When the same analysis was repeated on the intention-to-treat basis, the difference in PGI/II ratio between the intervention groups remained statistically significant (P=0.03).

The numbers of subjects with unchanged (seropositive to seropositive, seronegative to seronegative) or worsened (seronegative to seropositive) status of PG were 94 (90.4%) in the low-dose group and 96 (89.7%) in the high-dose group. When the low-dose group was considered as the reference group, the adjusted odds ratio (OR) of unchanged or worsened PG status for the high-dose group was 0.98 (95% confidence interval (95% CI) 0.4–2.5).

Discussion

About 10% of subjects converted to negative PG status in both groups, which may be explained to some extent by what is called 'regression to the mean,' including physiological fluctuation. Even in the absence of treatment, all follow up studies on chronic gastritis show apparent regression in a minority of subjects. In a large cohort follow up study in Colombia, 'regression' rates for the change from atrophy to normal (or superficial gastritis) and from intestinal metaplasia to atrophy were 7.5 and 4.4/100 person years, respectively.[11] The increase of PGII and decrease of PGI/II ratio in both groups contradict our hypothesis that vitamin C may be protective against the development of atrophy. When the change was compared between the two groups, the value was significant only for PGI/II ratio, while it failed to reach the level of statistical significance for PGII. This may be partially explained by the fact that PGI is relatively stable during aging, while PGII is known to increase with age.[12] In contrast, the difference of change in PGI/II ratio between the two groups remained statistically significant even after sex and age were controlled. Several interpretations may exist for the difference in the change of PGI/II ratio between the groups. The change, larger for PGI and smaller for PGII in the high-dose group compared to the low-dose group, became more distinct by taking the ratio of these variables (PGI as numerator and PGII as denominator), but it is not clear whether the difference in ratio, which is known to be related to corpus atrophy, reflects the characteristics of the subjects in this study, or is merely a chance finding. The PGI/II ratio was reported to show an association with severe corpus atrophy,[13] as well as hypochlorhydria.[14] This would be consistent with the high percentage of *H. pylori* infection, which is known to be related to distal gastric cancer, in the subjects in this study.[15] Also, gastric mucosal inflammation may have been reversed to some extent by the role of vitamin C in scavenging free radicals and protecting against lipid peroxidation.[3] Nevertheless, the possibility of a chance finding cannot be ruled out in view of the small number of subjects in both groups. Previous studies have failed to show any association between plasma vitamin C levels and PG levels.[16,17] It seems that while gastric juice vitamin C levels are associated closely with gastric pathology, the effect of serum vitamin C levels on PG levels is much smaller.

H. pylori infection is known to be related to gastric cancer.[15] On the other hand, both epidemiological and laboratory studies suggest that high intake of dietary vitamin C reduces the risk of gastric cancer.[1-3] Moreover, a significantly lower level of ascorbic acid in gastric juice has been reported in *H. pylori*-infected patients, and it has also been found that the value rises after eradication of *H. pylori*.[18, 19] We also measured serum IgG antibodies against Cag A. Cag A-positive *H. pylori* causes more extensive inflammation of the gastric mucosa,[20-22] which is more likely to progress to atrophic gastritis,[23] or gastric malignancy.[24] On this basis, one of the aims of this study was to evaluate the possible anti-*H. pylori* effect of vitamin C, as well as the change in Cag A status. However, the number of subjects whose status changed from seropositive to seronegative for *H. pylori* and Cag A was too small to allow detection of any association. The observed mild changes in *H. pylori* and Cag A status may be interpreted as contributing to the change in PGI/II ratio, rather than as reflecting any eradication effect due to vitamin C supplementation.

When the intention-to-treat cohort was set before amendment of the protocol, that is 217 subjects for the low-dose group and 222 subjects for the high-dose group, the conclusion was essentially the same, although the magnitude of the difference became smaller: the change of PGI/II ratio between baseline and after 5-year follow up was −0.19 for the low-dose group and −0.13 for the high-dose group (P=0.26). As nearly half of the subjects dropped out by the end of follow up from these groups, we considered it more appropriate to conduct the intention-to-treat analysis among subjects who remained after the protocol amendment.

The lack of a placebo arm may be critical to evaluate the supplemental effect of vitamin C. However, the mean dietary intakes of vitamin C were 150.9 mg, and 153.4 mg for the low-dose group and the high-dose group, respectively, as shown in Table 1. Thus, supplementation of 50 mg of vitamin C for the low-dose group may be interpreted as allowing this group to play a similar role to a placebo group. Further, the change of serum ascorbic acid level between baseline and after 5 years was statistically significantly different between the intervention groups (Table 2).

Although the difference in change of serum ascorbic acid was significantly different between the intervention groups, the observed changes in *H. pylori* infection status, Cag A status, and PG levels were rather moderate. It has been shown that in the presence of *H. pylori*-associated gastritis, the secretion of ascorbic acid from serum to stomach is severely impaired.[17] In this study, most of the subjects thus may not have had a high enough ascorbic acid level in the stomach to influence the seroprevalence of *H. pylori* or Cag A, or the level of PGs. According to Correa, ascorbic acid may mainly play a role at the phase from atrophic to metaplastic mucosa.[25] Thus, our findings do not conflict with the hypothesis that vitamin C is protective against gastric cancer.

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Food/nutrient intake and risk of atrophic gastritis among the *Helicobacter pylori*-infected population of northeastern Japan

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Although *Helicobacter pylori* (*H. pylori*) infection is considered a key risk factor for atrophic gastritis, along with other environmental factors, it is still unclear which factor is involved in the development of atrophic gastritis among *H. pylori*-infected subjects. In the present cross-sectional study, therefore, we analyzed various dietary factors in relation to the presence of atrophic gastritis among *H. pylori*-infected subjects who participated in a health check-up program in a town in northeastern Japan. One thousand and seventy-one subjects (362 males and 709 females) who provided both self-administered validated food frequency questionnaires and blood samples were the basis for the study, and all of them were serologically positive for *H. pylori* immunoglobulin G (IgG) antibody. Among them, 663 (223 males and 440 females) were diagnosed as having atrophic gastritis on the basis of serum pepsinogen levels. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated based on tertile categories of subjects without atrophic gastritis, using logistic regression analysis. Among females, high consumptions of rice (OR=1.6, 95% CI: 1.1–2.3), cod roe (OR=1.5, 95% CI: 1.0–2.2) and cuttlefish (OR=1.5, 95% CI: 1.0–2.3) were associated with a moderately increased risk of atrophic gastritis after adjustment for age (*P* for trend=0.02 for these items). Among males, high consumptions of rice and miso soup showed a tendency toward an increased risk (*P* for trend=0.12 and 0.13, respectively). Vegetables and fruits showed no association among either males or females. From these results, it is suggested that the dietary habits of consumers of traditional Japanese foods may play a role in the development of atrophic gastritis after *H. pylori* infection. (Cancer Sci 2003; 94: 372–377)

Atrophic gastritis is considered to be a pre-cancerous lesion of the stomach.[1–6] The relationship of gastric cancer with *Helicobacter pylori* (*H. pylori*) infection has been reported by many authors.[4,7] It is also known that *H. pylori* infection increases the risk of atrophic gastritis, although the relative risk varied in the range of 2–10.[4,8–16] These studies indicate that *H. pylori* may not be the sole cause of atrophic gastritis, because not all infected subjects eventually develop atrophic gastritis. Environmental factors, especially dietary intake, could also be important in explaining differences between subjects who do or do not develop atrophic gastritis.

Epidemiological studies investigating the association between environmental factors and atrophic gastritis have been limited, mainly because the proper diagnosis of atrophic gastritis earlier relied on endoscopic findings with/without biopsy, which is difficult to conduct outside a clinical setting. However, the serum levels of pepsinogen I (PG I) and pepsinogen II (PG II), together with the pepsinogen I/II ratio (PG I/II), have recently been used as markers for atrophic gastritis.[4,8–10,12,14,17] PG I/II in combination with PG I was demonstrated to be predictive of the histologic status of gastric mucosa,[18] and is considered as the most predictive marker for gastric cancer risk.[4,8–10,12,14,17] This method is less invasive than the endoscopic approach and is easy to use if blood samples are available, making it rather easy to employ in a community-based health check-up.

Previous epidemiological studies have suggested that not only *H. pylori* infection, but also a variety of environmental factors are important risk factors for atrophic gastritis,[4,8–16] e.g., low vegetable intake[9,12] and use of salt for seasoning.[19] However, food intake was assessed using frequency of food consumption only, and no studies have assessed food intake quantitatively with a validated questionnaire to evaluate the possible association between diet and atrophic gastritis. In the present study, therefore, we assessed the food intake quantitatively using a validated questionnaire, and evaluated the possible association between 20 dietary factors (especially vegetables and salty food intake) and the presence of atrophic gastritis diagnosed in terms of serum pepsinogen levels. Furthermore, which environmental factor is involved in the development of atrophic gastritis among persons infected with *H. pylori* has rarely been investigated. Therefore, we also investigated this point among *H. pylori*-infected subjects.

Subjects and Methods

Study subjects. The subjects were participants of a health check-up program in a town in northeastern Japan, where the mortality from gastric cancer is one of the country's highest. They were a subgroup of participants in the Japan Public Health Centers-based prospective study on cancer and cardiovascular diseases (JPHC Study) cohort I launched in 1990, and they were born between 1930 and 1949.[20] Self-administered food-frequency questionnaires (FFQ) were distributed through the public health centers to subjects on the occasion of a 5-year follow-up survey of the JPHC study in 1995. Of the 3399 subjects whose addresses were registered in the town as of 1990, 2872 (84.5%) filled out the questionnaire. Blood samples were obtained with written informed consent from 1566 (46.1%) subjects at the health check-up during that same year. A total of 1407 subjects (484 males and 923 females) who answered the self-administered questionnaires and provided blood samples were eligible.

Blood samples were tested for serum PG I and PG II as well as for *H. pylori* IgG antibody. Serum levels of PG I and PG II were used as a diagnostic tool for atrophic gastritis. Of 1407 eligible subjects, the analysis was restricted to 1141 (81.1%) whose *H. pylori* IgG antibody in the serum was positive.

Questionnaires. This self-administered questionnaire was composed of items such as general characteristics, the medical history of participants, cigarette smoking, alcohol consumption, vitamin supplement intake and dietary information. All subjects were asked about the average frequency of intake and portion

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size of 138 items over the past year.[21] Alcohol intake (g/day) of current drinkers was assessed based on reported consumption frequencies and amounts of five alcoholic beverages (sake, shochu, beer, whisky and wine). Daily consumption of rice was ascertained by categorized answers (numbers of rice bowls consumed per day: <1, 1, 2, 3, 4, 5, 6, 7–9, 10≤ bowls/day; the portion size: small, medium or large bowl). The frequency of miso soup consumption was classified into 6 categories (never, 1–3/month, 1–2, 3–4, or 5–6 per week and almost once/day), and the numbers of bowls per day were categorized in the same way as for rice intake. The frequency of other food items was classified into 9 categories (never, 1–3/month, 1–2, 3–4, or 5–6/week, almost once/day, 2–3, 4–6 or 7+ times/day), and the portion size was classified into 3 categories (more than 1.5 portion, same as the usual portion size, or less than half a portion). The intakes of sodium for each food were calculated using the food composition table developed for the food frequency questionnaire (FFQ) based on the Standard Tables of Food Composition in Japan, the 4th revised edition.[22] The salt intake from cooking salt and salty seasonings such as soy sauce was estimated from the cooking methods frequently used for meats, fish and vegetables.[23] To confirm the validity of this questionnaire, we collected questionnaires and 28-day dietary records (7 days in each of the four seasons) from 215 subjects in four areas, and estimated the amount of intake per food group from them. Spearman correlation coefficients for the intakes of the fish group, vegetable group, and fruit group and sodium from the questionnaire and those from the dietary record were 0.46, 0.33, 0.61 and 0.59 in males, and 0.42, 0.35, 0.50 and 0.55 in females, respectively.[24] The validity indices of food items that were consumed less frequently were inevitably unreliable against dietary records over a short duration, and are not listed here. We concluded that this questionnaire had reasonable validity for use in large-scale epidemiological studies.

H. pylori. *H. pylori* infection was determined by measuring serum *H. pylori* IgG antibodies. Specific anti-*H. pylori* antibodies in sera stored at −80°C were measured with an enzyme-linked immunosorbent assay (ELISA) kit using an acid-extracted antigen (Helico G, Porton Cambridge, Oxford, UK). The sensitivity and specificity of the assay was 96% and 86%, respectively, compared with gastric biopsy findings.[25]

Criteria for atrophic gastritis. The serum levels of PG I and PG II were measured by radioimmunometric assay kits (PG I/PG II RIABEAD, Dainabot Co., Ltd., Tokyo) at an outside laboratory (SRL, Inc., Tokyo). Atrophic gastritis was diagnosed according to two criteria: a PG I level below 70 ng/ml and a PG I/II ratio below 3.0. This was because these criteria show a high geographic correlation with the age-adjusted mortality rate of gastric cancer among five Japanese populations (r=0.99) and have the best predictive ability for gastric cancer mortality.[17] Recent studies have adopted these criteria, and their reliability has been recognized.[4, 8–10, 12, 14]

Data analysis. Three people with a prior history of gastrectomy were excluded from the study along with three others with a history of gastric cancer. Fifty-five subjects with energy intakes that were unreasonably high (≥4000 calories/day for males; ≥3600 calories/day for females) or low (<900 calories/day for males; <800 calories/day for females), or thirteen subjects who failed to answer questions about dietary intake, were also excluded from the analysis (n=64). In this study, we analyzed data from 1071 eligible subjects (362 males and 709 females) and examined the possible association between serologically determined atrophic gastritis and food intake among subjects with *H. pylori* infection.

For statistical analysis, the total intake of each food was divided into 3 categories at the nearest tertile based on the distribution in the control group. As regards smoking habits, subjects were classified as never, past, or current smokers. As for alcohol use, subjects were classified as non-drinkers or drinkers consuming <50 or 50≤ g per day. The trend was assessed by assigning ordinal values for categorical variables and median values for continuous variables.

Table 1. Characteristics of serologically determined atrophic gastritis cases and controls

Characteristics	Males			Females		
	AG[1] n=223	NAG[2] n=139	P for difference	AG[1] n=440	NAG[2] n=269	P for difference
Age	57.5 (0.4)	55.0 (0.5)	<0.0001	57.1 (0.3)	55.8 (0.3)	0.001
Cigarette smoker (%)[3]	47.1	39.6	0.16	2.1	2.6	0.63
Alcohol (g/day)	30.8 (1.9)	31.1 (2.4)	0.90	0.6 (0.1)	0.8 (0.2)	0.38
Total fish (g/day)	110.7 (4.8)	116.3 (6.1)	0.47	110.4 (3.1)	104.7 (4.0)	0.26
Salted fis >1/week (%)	61.0	66.9	0.26	68.6	66.5	0.56
Dried fis >1/week (%)	27.4	25.9	0.76	27.5	33.1	0.11
Cod roe >1/week (%)	64.1	60.4	0.48	58.2	48.0	0.01
Cuttlefish >1/week (%)	41.7	38.1	0.50	38.0	29.0	0.01
Meat (g/day)	58.7 (3.1)	71.8 (3.9)	0.01	55.6 (2.2)	59.3 (2.8)	0.29
Rice (g/day)	366.3 (8.7)	361.7 (11.0)	0.75	287.0 (5.0)	276.7 (6.3)	0.20
Fruit (g/day)	201.6 (12.1)	175.6 (15.3)	0.18	274.8 (10.6)	260.9 (13.5)	0.42
Green-yellow vegetables (g/day)	68.7 (3.6)	65.7 (4.6)	0.61	84.7 (3.2)	79.5 (4.1)	0.32
Broccoli >1/week (%)	46.2	48.9	0.61	62.7	70.6	0.03
Other vegetables (g/day)	130.9 (9.3)	122.9 (11.8)	0.60	143.2 (4.7)	135.5 (6.0)	0.31
Cabbage <1/week (%)	87.4	92.1	0.17	92.1	91.8	0.92
Pickled vegetables (g/day)	57.1 (4.1)	55.1 (5.2)	0.75	63.6 (2.8)	65.0 (3.6)	0.76
Soybean products (g/day)	113.5 (4.3)	110.7 (5.4)	0.68	108.0 (3.2)	112.2 (4.1)	0.42
Miso soup (g/day)	414.7 (10.8)	381.3 (13.7)	0.06	318.9 (7.0)	311.9 (9.0)	0.53
Sodium (mg/day)	6384.8 (185.7)	6415.4 (235.2)	0.92	6245.6 (122.3)	6134.9 (156.5)	0.58
Carotene (μg/day)	3036.1 (144.5)	2829.7 (183.1)	0.38	3642.0 (127.0)	3400.1 (162.7)	0.24
Vitamin C (mg/day)	153.9 (6.3)	149.3 (8.0)	0.65	193.6 (5.3)	178.4 (6.8)	0.08
Calcium (mg/day)	579.9 (18.2)	586.3 (23.0)	0.83	592.3 (13.6)	599.7 (17.4)	0.74

Based on Student's t test or χ^2 test.
Figures are means (SE) unless otherwise specified.
1) AG=atrophic gastritis. *2)* NAG=non-atrophic gastritis. *3)* Current male smokers, current and past female smokers.

Student's t test or the χ^2 test was used to compare the characteristics of subjects with and without atrophic gastritis. Odds ratios (ORs) and their 95% confidence intervals (95% CI) were obtained by logistic regression analysis. The ORs of smoking and alcohol consumption were compared with those of never smokers and non-drinkers, respectively, for reference. As for the other foods, the ORs were compared with the lowest tertile of intake as the reference. The dose-response association was tested by a χ^2 estimate of the linear trend in the medians of each category. Two-sided P values <0.05 were considered statistically significant. All statistical analyses were performed using the SAS statistical software package.[26]

Results

Of 1071 subjects with *H. pylori* infection, 663 were diagnosed with serologically determined atrophic gastritis. The overall prevalence of atrophic gastritis was 61.9% (male 61.6%, female 62.1%).

Table 1 shows the characteristics and distribution of dietary factors among subjects with *H. pylori* infection. The average age of subjects with and without atrophic gastritis was 57.5 and 55.0 in males, and 57.1 and 55.8 in females, respectively. Among females, subjects with atrophic gastritis had a higher consumption of cod roe and cuttlefish than subjects without it (P<0.05). On the contrary, the proportion of subjects with atrophic gastritis who consume broccoli one or more times per week was lower than in subjects without atrophic gastritis (P<0.05). The average consumption of miso soup tended to be lower in male subjects with atrophic gastritis compared to those without it. No substantial differences were observed for the intake of other foods between subjects with and without atrophic gastritis.

The analysis of cigarette smoking or alcohol use in the present study was restricted to males. The crude OR for current smokers was 1.2 (95% CI: 0.7–1.9) compared with never smokers. Current smoking statistically significantly increased the OR of atrophic gastritis with the adjustment for age (OR=1.7, 95% CI: 1.0–2.9). There was no clear association between total alcohol use and atrophic gastritis. The effect on atrophic gastritis did not differ by alcohol beverage type. Smoking and drinking risks for atrophic gastritis were not evaluated among females because female smokers and drinkers were so few in this study population. When we estimated ORs of atrophic gastritis for each food, we adjusted for smoking status only in the analysis for males.

Table 2 shows the ORs of serologically determined atrophic gastritis in relation to sodium and some traditional Japanese foods including rice, fish and miso soup. Among females, an increased OR was observed for the highest consumption category of both cod roe (OR=1.5, 95% CI: 1.0–2.2) and cuttlefish (OR=1.5, 95% CI: 1.0–2.3) after adjustment for age. We found a steady increase in the OR of atrophic gastritis as the intake of rice increased (OR for intermediate tertile=1.3, high tertile=1.6, P for trend<0.05) after adjustment for age. After a further adjustment for age and cod roe, high consumption of rice statistically significantly increased the OR of atrophic gastritis (P for trend<0.05). Although the estimated OR for the intermediate tertile of sodium intake was 1.9 (95% CI: 1.3–2.7) compared with the low tertile, sodium intake did not show a statistically significant trend. High consumption of dried fish tended to decrease the OR for atrophic gastritis.

Among males, no notable association was observed between atrophic gastritis and various types of fish intake after adjustment for age and smoking status. High consumption of miso soup and rice was associated with a slightly increased OR of atrophic gastritis but without statistical significance (miso soup: for high tertile, OR=1.6, 95% CI: 0.9–2.8, trend P=0.13, rice: for high tertile, OR=1.6, 95% CI: 0.9–2.9, trend P=0.12).

Table 3 shows the results of multivariate analysis (including selected vegetables and fruits, which affected the OR of atrophic gastritis in univariate analysis). Among both males and fe-

Table 2. Crude and adjusted odds ratio (OR) and 95% confidence interval (CI) for serologically determined atrophic gastritis among subjects with *H. pylori* infection

Food items		Males						Females					
		Median	AG[1] *n*	NAG[2] *n*	Adjusted OR[3]	95% CI	*P* for trend	Median	AG[1] *n*	NAG[2] *n*	Adjusted OR[4]	95% CI	*P* for trend
Salted fish	Low	4.7	87	46	1.0			4.7	138	90	1.0		
	Intermediate	15.0	76	53	0.7	0.4–1.1		15.0	174	109	1.0	0.7–1.5	
	High	35.0	60	40	0.7	0.4–1.2	0.28	35.0	128	70	1.2	0.8–1.7	0.45
Dried fish	Low	0	77	41	1.0			0	153	81	1.0		
	Intermediate	3.3	83	60	0.6	0.4–1.1		3.3	166	99	0.9	0.6–1.4	
	High	10.7	63	38	0.9	0.5–1.5	0.85	10.7	122	89	0.7	0.5–1.1	0.11
Cod roe	Low	0.7	77	53	1.0			0.7	111	75	1.0		
	Intermediate	4.3	86	45	1.3	0.8–2.2		1.3	130	96	1.0	0.7–1.5	
	High	10.0	60	41	1.0	0.6–1.8	0.93	5.0	199	98	1.5	1.0–2.2	0.02
Cuttlefish	Low	3.3	86	57	1.0			1.7	84	56	1.0		
	Intermediate	5.0	83	52	1.1	0.6–1.7		3.3	189	135	1.0	0.7–1.5	
	High	16.1	55	30	1.1	0.6–2.0	0.76	10.7	167	78	1.5	1.0–2.3	0.02
Miso soup	Low	150	34	30	1.0			150	136	83	1.0		
	Intermediate	300	55	36	1.3	0.7–2.6		300	160	100	1.0	0.7–1.4	
	High	450	134	73	1.6	0.9–2.8	0.13	450	144	86	1.0	0.7–1.5	0.90
Rice	Low	247.4	57	46	1.0			202.0	122	90	1.0		
	Intermediate	339.8	85	47	1.6	0.9–2.8		247.4	146	90	1.3	0.9–1.8	
	High	472.8	81	46	1.6	0.9–2.9	0.12	354.7	172	89	1.6	1.1–2.3	0.02
Sodium	Low	4040.1	71	46	1.0			3700.1	107	90	1.0		
	Intermediate	5853.8	69	46	1.0	0.5–1.6		5763.3	198	90	1.9	1.3–2.7	
	High	8774.2	83	47	1.1	0.6–1.9	0.73	8454.7	135	89	1.3	0.9–2.0	0.30

The total intake of each food was divided into 3 groups (low tertile, intermediate tertile, or high tertile) based on the distribution in the control group.
Calculated by logistic analysis.
1) AG=atrophic gastritis. *2*) NAG=non-atrophic gastritis. *3*) Adjusted for age and smoking. *4*) Adjusted for age.

Table 3. Crude and adjusted odds ratio (OR) and 95% confidence interval (CI) for serologically determined atrophic gastritis among subjects with *H. pylori* infection

Food items		Males						Females					
		Median	AG[1] *n*	NAG[2] *n*	Adjusted OR[3]	95% CI	*P* for trend	Median	AG[1] *n*	NAG[2] *n*	Adjusted OR[4]	95% CI	*P* for trend
Green-yellow vegetables	Low	26.8	78	46	1.0			30.4	134	90	1.0		
	Intermediate	54.3	62	47	0.7	0.4–1.3		62.6	142	89	1.0	0.7–1.5	
	High	107.1	83	46	1.0	0.6–1.7	0.87	122.7	164	90	1.2	0.8–1.7	0.38
Other vegetables	Low	56.9	79	46	1.0			60.8	147	89	1.0		
	Intermediate	104.2	61	47	0.7	0.4–1.3		115.4	137	90	0.9	0.6–1.3	
	High	188.9	83	46	1.0	0.6–1.8	0.72	214.3	156	90	1.1	0.7–1.6	0.59
Pickled vegetables	Low	17.1	80	47	1.0			18.6	143	89	1.0		
	Intermediate	36.3	58	46	0.7	0.4–1.3		42.3	152	90	1.0	0.7–1.5	
	High	87.0	85	46	1.0	0.6–1.6	0.93	112.5	145	90	1.0	0.7–1.5	0.98
Fruits	Low	51.2	87	47	1.0			96.4	132	89	1.0		
	Intermediate	136.7	56	46	0.7	0.4–1.3		205.3	144	90	1.1	0.7–1.6	
	High	334.0	80	46	1.0	0.6–1.8	0.73	407.0	164	90	1.2	0.8–1.8	0.32
Broccoli	Low	0.7	66	36	1.0			1.4	188	98	1.0		
	Intermediate	1.4	64	40	0.8	0.4–1.5		4.5	116	78	0.8	0.5–1.1	
	High	5.3	93	63	0.8	0.5–1.4	0.63	10.5	136	93	0.8	0.6–1.1	0.25
Cabbage	Low	5.9	71	38	1.0			5.9	141	90	1.0		
	Intermediate	13.8	71	45	0.9	0.5–1.6		13.8	149	99	1.0	0.7–1.4	
	High	20.7	81	56	0.7	0.4–1.3	0.28	32.5	150	80	1.2	0.8–1.8	0.30

The total intake of each food was divided into 3 groups (low tertile, intermediate tertile, or high tertile) based on the distribution in the control group.
Calculated by logistic analysis.
1) AG=atrophic gastritis. *2*) NAG=non-atrophic gastritis. *3*) Adjusted for age and smoking. *4*) Adjusted for age.

males, neither vegetables nor fruits demonstrated an inverse trend. Intake of pickled vegetables, a diet high in salt, did not show any association with atrophic gastritis. No association of vitamin C or carotene with atrophic gastritis was observed.

Discussion

The present study focused on the food intake of subjects with *H. pylori* infection. It contributed to clarification of the association between various dietary factors and atrophic gastritis after *H. pylori* infection.

A positive association between traditional Japanese food (rice and cod roe) and atrophic gastritis in females was clearly observed in this study. In males, rice and miso soup were associated with an increased risk for atrophic gastritis, although this was not statistically significant. The result that a high consumption of cod roe or miso soup was associated with an increased risk of atrophic gastritis may be regarded as supportive of the association between salt intake and atrophic gastritis, because cod roe and miso soup are generally salt-rich. Our study showed a trend of increasing risk of atrophic gastritis with the consumption of rice in females. The association between rice intake and gastric cancer has been inconsistent, with some studies showing a positive association,[3,27–29] and others finding none at all.[6,30] The results from the previous epidemiological studies are likewise inconsistent regarding the association between rice and atrophic gastritis. One study demonstrated a severe reduction in the PG I/II ratio with high rice/pasta consumption in Venezuela,[31] whereas another reported a decreased risk of atrophic gastritis, but an increased risk of gastric cancer from rice intake.[3] The mechanism by which a high consumption of rice increases the risk of gastric cancer is unclear. Ji *et al.* suggested that carbohydrate could possibly irritate the gastric mucosa physically (especially in the form of rough whole-grain cereals).[32] Although rice is a typical source of carbohydrate, it is unclear whether or not it physically affects the mucosa. An indirect effect of rice intake on atrophic gastritis may exist. Rice may correlate with other dietary factors and it may act as a marker of intake of traditional Japanese foods or salted foods.

As to fish consumption (including salted and dried fish), we did not find any association with atrophic gastritis, in contrast to a previous experimental study showing that fish meal enhanced *H. pylori*-induced gastritis in Mongolian gerbils.[33] Fish meal might contain factors, other than salt, that greatly enhance *H. pylori*-induced gastritis. In another epidemiological study, traditional Japanese foods (rice, miso soup, raw fish and cod roe) increased the risk of gastric cancer.[29] It was suggested that such foods might accelerate the development of atrophic gastritis and gastric cancer, though the mechanisms involved need to be clarified.

The significant increase in the risk of atrophic gastritis associated with high cuttlefish consumption was unexpected. If the cuttlefish is in the form of "shiokara" (salted cuttlefish, a traditional Japanese food), it might increase the risk of atrophic gastritis, although data on the cooking methods were not available from this questionnaire. No other epidemiological or experimental studies have shown such a positive association between cuttlefish and atrophic gastritis. This result may well be only a chance finding. As in the case of cuttlefish, we can not deny that the positive association between cod roe and atrophic gastritis may be coincidental. However, although cod roe is a minor contributor of salt/sodium in terms of its portion size and intake frequency, it contains a high concentration of sodium per serving. Therefore, it is possible that cod roe is a risk factor for atrophic gastritis.

We could not detect a statistically significant association between atrophic gastritis and any food item in males, though some food items (rice, cod roe and cuttlefish) were associated with atrophic gastritis in females. The negative results for several food items among males must be interpreted with caution because of the small number of male subjects. One possibility is that the effect may be truly confined to females. Alternatively, it may be owing to the residual confounding effect of smoking and dietary factors in males.

If high consumption of sodium indeed affects the development of atrophic gastritis, the lack of a positive association between atrophic gastritis and salted fish or pickled vegetables

would be unexpected. But there are two possible interpretations of this lack of an association. First, our study subjects were residents in the high-risk area in Japan, and there was a possibility that they had homogeneous dietary habits, making it difficult to detect such a positive association. Second, it might be difficult to identify risk factors in recent dietary habits, if dietary risk factors, especially in early life, play a more important part in the development of atrophic gastritis as Kato *et al.* suggested.[34]

High salt intake and its association with gastric cancer have been reported in many studies.[5, 30, 35] Sodium chloride exerts a dose-dependent, tumor-promoting activity on gastric carcinogenesis in rats when given after N-methyl-N′-nitrosoguanidine.[36] Several epidemiological studies have reported an increased risk for atrophic gastritis associated with high salt intake[37] or salt seasoning.[19] In our study, the adjusted OR for the intermediate tertile of sodium intake compared to the low tertile was 1.9, whereas sodium intake did not show any significant trend in females, and no association at all was observed in males. The lack of a significant positive association with sodium intake could be due to three reasons, one related to dietary assessment, another to the stage of atrophic gastritis development and the third, to the range of individual consumption of sodium. First, a dietary assessment of sodium may not be appropriate. We compared the intake levels of sodium assessed with this questionnaire and two corresponding 24-h urinary excretion levels in residents of 3 JPHC study areas including the present study area. The Spearman rank correlation coefficients between dietary sodium assessed with this questionnaire and the urinary excretion data were weak or null; 0.24 and −0.10 in males and females, respectively,[38] although 24-h urinary excretion is not necessarily a reliable biomarker, due to day-to-day variation. Sodium intake is from consumption of miso soup (20%), fish and shellfish (16%) and pickled vegetables (12%). Seasonings such as table salt and soy sauce are major contributors of sodium intake (approximately 30%). However, the amount of sodium intake from such seasonings could not be assessed by this FFQ.[39] The Spearman correlation coefficients for sodium intakes from the questionnaire and those from the dietary record were moderate (r=0.59 in males, r=0.55 in females) across four areas with a relatively large range of variation; however, they may be relatively low only in this study area. Additionally, our previous study suggested that even though the sodium concentration in the food items is different, the uniform composition table gives the same sodium value, making exact evaluation impossible.[40] Therefore, the fact that it is somewhat difficult to quantify the intake of sodium using this questionnaire may explain in part the absence of any association between sodium intake and gastritis in this study. Intake of highly salted food such as cod roe may be a more reliable marker for dietary sodium intake. Second, an experimental study has reported that excessive NaCl intake enhances *H. pylori* colonization in mice and in humans and that chronic salt intake may exacerbate gastritis by increasing colonization.[41] In this previous experimental study, no significant exacerbation of inflammation was associated with high salt intake among *H. pylori*-infected mice. In the light of this result, high salt intake may not be associated with development of atrophic gastritis after *H. pylori* infection. Third, the mean intake of sodium was relatively high, and the standard deviation was within narrow limits in this study area compared with that in 3 other areas in Japan.[23] This may make it difficult to detect a positive association.

Many studies have suggested that the intake of vegetables and fruits plays a protective role against gastric cancer.[34, 42] Vegetables/fruits are rich in micronutrients, especially vitamin C, which may inhibit the endogenous formation of carcinogenic nitrosamines.[43] Some researchers reported that vegetables and fruits protected against atrophic gastritis,[9, 12, 31] while others found no such association.[3, 6, 8, 10, 11, 13, 16] Our results revealed no association in either males or females. One possible reason for the apparent lack of a protective association is that vegetable/fruit intake may have no effect on the development of atrophic gastritis, although it may influence carcinogenesis in the stomach. Another may be the relatively low green and yellow vegetable intake within this region compared with other Japanese areas.[44] In other words, the consumption level of green-yellow vegetables even in the highest tertile group may not be high enough to prevent atrophic gastritis.

Of 3399 residents selected from the study area, 1407 (41.4%) who provided both questionnaires and blood samples proved to be eligible. Thus, the proportion of subjects with available information was not very high, although it was comparable with that in other studies of this kind. Though the low response rate could have introduced selection bias, both questionnaires and blood samples were collected independently, and thus we believe bias should be negligible. Information bias, particularly recall bias, needs consideration, since the questionnaire was self-administered, and subjects were asked about their dietary intake over the past year. In a correlation analysis of dietary intakes estimated from the questionnaire with those from the 28-day dietary record, we confirmed that this questionnaire has reasonable validity for use in epidemiological studies. *H. pylori* infection and atrophic gastritis were classified using biological tests to minimize misclassification. Although the possibility of misclassification of both exposure and outcome status cannot be excluded, it would be non-systematic, and the estimates of the association presented here would be distorted towards the null, and therefore would represent conservative estimates of the effects.

Because of our analysis by cross-sectional design, we cannot rule out the possibility that the diet developed atrophic gastritis, or that the presence of atrophic gastritis leads to changes in the dietary pattern; in other words, a causal link cannot be established by the present study.

Allowing for these methodological issues, our results indicate that traditional Japanese food, including rice and cod roe, appears to moderately increase the risk for atrophic gastritis. Most epidemiological studies (including ours) dealing with the association between food intake and atrophic gastritis were conducted as cross-sectional or case-control studies. A prospective study will be necessary to clarify the risk factors for causing atrophic gastritis.

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Original Research

Effect of Five-Year Supplementation of Vitamin C on Serum Vitamin C Concentration and Consumption of Vegetables and Fruits in Middle-Aged Japanese: A Randomized Controlled Trial

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Key words: vitamin C supplementation, serum vitamin C, fruit and vegetable intake, randomized controlled trial, Japanese

Objective: This study was aimed at evaluating the effect of long-term vitamin C supplementation on serum and dietary vitamin C and identifying the factors associated with change in serum concentration.

Methods: A total of 439 subjects with atrophic gastritis initially participated in a randomized clinical trial using vitamin C and β-carotene to prevent gastric cancer. We originally randomized the participants into four treatment groups using a 2×2 factorial design, whereby 0 or 15 mg/day β-carotene and 50 or 500 mg/day vitamin C were administered in a double-blind manner. The β-carotene component was terminated early after a mean treatment duration of four months. Before and upon early termination of β-carotene supplementation, 134 subjects dropped out this trial, while 120 and 124 subjects took the vitamin C supplement at either 50 mg or 500 mg daily for five years.

Results: Changes in serum vitamin C were significantly higher in the high-dose group (38.5% increase, 95% CI = 27.0–49.9) than in the low-dose group (13.0% increase, 5.1–20.9) or in the dropout group (3.3% increase, −2.1–8.6) after five-year supplementation. The serum vitamin C at baseline was negatively associated with changes in serum vitamin C ($p < 0.0001$), while high-dose ($p < 0.0001$) and low-dose ($p < 0.05$) supplementation and female gender ($p < 0.001$) were positively associated. Dietary intake of vitamin C in the supplementation group was almost identical before and after five-year supplementation of vitamin C (2.31 mg/day decrease, 95% CI = −15.3–10.7), while a 17.7 mg/day decrease (95% CI = −44.2–8.86) was observed in the drop-out group.

Conclusion: Five-year vitamin C supplementation induces a remarkable increase in serum vitamin C concentration, and our intervention program appears to have no effect on dietary vitamin C intake.

INTRODUCTION

For the last several decades a number of epidemiologic studies have shown that diets high in vegetables and fruits and/or high blood concentration of antioxidant vitamins may protect against cancers of the mouth, thorax, esophagus, lung, stomach, colon and rectum [1–4]. Several large-scale chemoprevention trials have also been conducted in China, Finland and the United States to test the efficacy of retinal β-carotene, α-tocopherol and other nutrients [5–7]. There is considerable controversy as to harmful effect of β-carotene and cancer risk [7], whereas epidemiologic evidence, although not completely consistent, has shown that vitamin C, vitamin E and selenium are associated with a decreased cancer risk.

The plasma level of vitamin C and carotenoids may serve as biomarkers of consumption of vegetables and fruits that are the primary dietary sources of these vitamins [8–10]. In our previous study [11], an increase of serum vitamin C after three-month

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