TABLE IV – MULTIVARIATE RATE RATIOS OF GASTRIC CANCER WITH 95% CONFIDENCE INTERVALS ACCORDING TO QUARTILE OF THE 3 MAJOR DIETARY PATTERNS, JPHC STUDY, 1990–1999

	Quartiles (male)				*p* for trend	Quartiles (female)				*p* for trend
	1 (low)	2	3	4 (high)		1 (low)	2	3	4 (high)	
Healthy dietary pattern										
Total gastric cancer										
Person years	47,265	47,481	47,622	47,710		52,124	52,338	52,530	52,289	
Cases	57	66	74	88		36	23	32	24	
RR (95% CI)	1.00	1.00	1.06	1.13	0.39	1.00	0.57	0.77	0.56	0.03
		(0.70–1.45)	(0.74–1.53)	(0.78–1.63)			(0.33–0.97)	(0.47–1.26)	(0.32–0.96)	
Differentiated type										
Cases	36	38	43	57		12	7	12	9	
RR (95% CI)	1.00	0.91	0.97	1.11	0.29	1.00	0.53	0.81	0.62	0.31
		(0.57–1.45)	(0.61–1.53)	(0.70–1.76)			(0.21–1.34)	(0.35–1.88)	(0.25–1.54)	
Undifferentiated type										
Cases	17	27	22	27		21	14	19	12	
RR (95% CI)	1.00	1.37	1.10	1.24	0.71	1.00	0.57	0.79	0.46	0.04
		(0.73–2.58)	(0.57–2.15)	(0.64–2.40)			(0.29–1.15)	(0.42–1.49)	(0.22–0.96)	
Traditional dietary pattern										
Total gastric cancer										
Person years	46,883	47,043	47,711	48,441		51,611	52,011	52,487	53,172	
Cases	30	62	81	112		17	30	23	45	
RR (95% CI)	1.00	1.97	2.47	2.88	< 0.0001	1.00	1.70	1.28	2.40	0.007
		(1.25–3.12)	(1.55–3.94)	(1.76–4.72)			(0.93–3.12)	(0.68–2.44)	(1.32–4.35)	
Differentiated type										
Cases	20	41	50	63		5	9	10	16	
RR (95% CI)	1.00	2.05	2.37	2.67	< 0.0001	1.00	1.82	1.79	2.40	0.06
		(1.16–3.62)	(1.32–4.28)	(1.42–5.02)			(0.60–5.49)	(0.60–5.39)	(0.83–6.97)	
Undifferentiated type										
Cases	7	19	23	44		11	16	12	27	
RR (95% CI)	1.00	2.50	3.27	4.92	0.006	1.00	1.32	1.03	2.31	0.03
		(1.02–6.12)	(1.32–8.12)	(1.92–12.6)			(0.60–2.89)	(0.45–2.37)	(1.09–4.89)	
Western dietary pattern										
Total gastric cancer										
Person years	47,649	47,862	47,309	47,258		52,618	52,302	52,274	52,086	
Cases	83	77	64	61		32	27	27	29	
RR (95% CI)	1.00	0.99	0.88	0.85	0.45	1.00	0.94	0.92	1.13	0.42
		(0.71–1.37)	(0.63–1.24)	(0.60–1.38)			(0.56–1.57)	(0.54–1.56)	(0.66–1.93)	
Differentiated type										
Cases	48	46	44	36		12	7	13	8	
RR (95% CI)	1.00	1.07	1.07	0.88	0.45	1.00	0.67	1.23	0.86	0.68
		(0.71–1.62)	(0.70–1.64)	(0.56–1.38)			(0.26–1.70)	(0.54–2.77)	(0.34–2.22)	
Undifferentiated type										
Cases	30	24	18	21		17	15	14	20	
RR (95% CI)	1.00	0.78	0.64	0.78	0.96	1.00	0.96	0.87	1.40	0.30
		(0.44–1.37)	(0.35–1.17)	(0.44–1.40)			(0.48–1.93)	(0.42–1.81)	(0.70–2.78)	

Multivariate adjustment included age (as a continuous variable), body mass index (as a continuous variable), energy intake (as a continuous variable), education level, family history of gastric cancer (yes, no), smoking status (for males) and alcohol drinking (for males).

The possibility of histologic misclassification cannot be ruled out. Histologic typing is inherently subjective, lacks gold-standard measures and depends on the judgment of pathologists.[43] Furthermore, a review of pathologic slides by even one pathologist in a subsample was not practical in this large multicenter study. This limitation may have led to histologic misclassification to some extent and resulted in the absence of any difference in the findings according to the histologic subtypes.

Factor analysis is inherently arbitrary and subjective on the selection of included variables, the number of retained factors, the method of rotation and the labeling of identified factors.[15,44] We repeated the same analyses with varying numbers of factors and when randomly dividing the sample into 2 groups (both in total subjects and for each gender) to examine whether these subjective choices affected the reproducibility of our findings. The results showed very similar dietary patterns. Also, we adhered as closely as possible to the established empirical guidelines for the principal component method of factor analysis. Moreover, the dietary patterns defined in this analysis were not established *a priori* but based on the actual data. The nutritional implications of the 3 dietary patterns were generally understandable.

The dietary pattern approach using factor analysis is population-dependent and may differ according to the geographic area, race, culture, *etc.* Hence, the generalizability of the results of this method is a critical concern. The fact is, different investigations within the same[26,27] or even various populations[16,17,19] generated similar dietary patterns.

In conclusion, the major dietary patterns of the Japanese were identified using factor analysis, and the present findings indicated that the healthy pattern decreased the risk of gastric cancer, while the traditional pattern increased the risk of gastric cancer.

ACKNOWLEDGEMENTS

M.K.K. was awarded a Visiting Scientist Fellowship from the Foundation for Promotion of Cancer Research in Japan.

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APPENDIX

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Gastric Cancer (2004) 7: 46–53
DOI 10.1007/s10120-004-0268-5

Original article

Association of *Helicobacter pylori* infection and environmental factors in non-cardia gastric cancer in Japan

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Abstract

Background. **Although *Helicobacter pylori* infection is a major risk factor for gastric cancer, it does not explain the full picture of stomach carcinogenesis. There have been few epidemiological studies, however, which examined both *H. pylori* and environmental factors simultaneously. The aims of this study were to estimate the association of environmental factors (smoking and dietary factors) with gastric cancer in consideration of *H. pylori* infection, and to investigate the effects of the interaction between environmental factors and *H. pylori* infection.**

Methods. **A multicenter, hospital-based, case-control study of gastric cancer was conducted at four hospitals in Nagano prefecture, Japan, between October 1998 and March 2002. For 153 newly diagnosed gastric cancer cases, two controls matched by age (within 3 years), sex, and residence area were randomly selected from the participants of a health check-up program during the same period in the same hospitals. We conducted a questionnaire survey and obtained blood samples. Consequently, 122 non-cardia gastric cancer cases and 235 controls were available for this analysis.**

Results. ***H. pylori*** **infection was strongly associated with non-cardia gastric cancer after adjustment for possible confounding factors (odds ratio [OR], 8.2; 95% confidence interval [CI], 3.7–18.2). Cigarette smoking (OR, 2.8; 95% CI, 1.2–6.5) and frequent intake of miso (fermented soy bean) soup (OR, 2.1; 95% CI, 0.9–5.1) and rice (OR, 2.5; 95% CI, 1.0–6.1) were determined to be risk factors even after adjusting for possible confounding factors, including *H. pylori* infection. However, no statistically significant interaction between environmental factors and *H. pylori* infection was detected.**

Conclusion. **This finding suggests that although *H. pylori* infection is clearly an important risk factor for gastric cancer, smoking cessation and dietary modification may be practical strategies for the prevention of non-cardia gastric cancer among both *H. pylori*-positive and -negative subjects in Japan.**

Key words *Helicobacter pylori* · Gastric cancer · Smoking · Diet · Case-control study

Offprint requests to: S. Tsugane
Received: August 12, 2003 / Accepted: January 5, 2004

Introduction

In 1994, the International Agency for Research on Cancer recognized *Helicobacter pylori* as a class I human carcinogen. A combined analysis of 12 case-control studies nested within prospective cohorts suggested that the odds ratio (OR) for the association between *H. pylori* infection and gastric cancer was 2.36 (95% confidence intervals [CIs], 1.98–2.81) [1]. Thus, there is a strong link between *H. pylori* and gastric cancer in many countries. By contrast, low gastric cancer rates have been reported in some countries with a high prevalence of *H. pylori* infection, such as India and Bangladesh [2]. This difference in gastric cancer rates in populations with similar high prevalences of *H. pylori* infection could be related to the difference in the diversity of *H. pylori* strains, ethnicity, and environmental factors.

There is recently increasing evidence that *H. pylori* strains that possess the cytotoxin-associated gene A (CagA) are associated with an increased risk of atrophic gastritis and gastric cancer. CagA-positive *H. pylori* infection strongly increased the risk for gastric cancer compared with *H. pylori*-uninfected subjects [3–6].

In a case-control study in Brazil, Tatemichi et al. [7] showed there were ethnic differences in the strength of the association between CagA serological status and non-cardia gastric cancer, which suggested that environmental factors played an important role in gastric car-

cinogenesis in Japanese Brazilians compared to non-Japanese Brazilians.

A relationship between environmental factors and gastric cancer has been reported in numerous previous studies [8–12]. A low intake of vegetables and a high intake of salt and salty foods were considered to be risk factors for gastric cancer [10,11]. Our previous study showed the protective effect of mushrooms and cruciferous vegetables among the same subjects as those of this present study, although we did not take into account *H. pylori* infection [12]. Moreover, there have been few epidemiological studies that have investigated the association between environmental factors and gastric cancer in consideration of *H. pylori* infection, and the interaction between environmental factors and *H. pylori* infection [13–16].

Concerning anatomical subsites, gastric cancer has been divided into two groups: "cardia" cancer (the upper third around the cardia) and "non-cardia" cancer (middle body and antrum). In the study by Hansen et al. [17], *H. pylori* infection was found to be associated with an increased risk of non-cardia cancer, but had an inverse association with cardia cancer. Our study subjects were restricted to cases of non-cardia cancer, which had a strong association with *H. pylori* infection.

The aim of this study was not only to confirm the association between non-cardia gastric cancer and *H. pylori* infection, combined with CagA, but also to estimate the association of environmental factors (smoking and dietary factors) with non-cardia cancer in consideration of *H. pylori* infection, which is a prevalent risk factor. We also investigated the effects of the interaction between environmental factors and *H. pylori* infection.

Subjects and methods

Subjects

A multicenter, hospital-based, case-control study of stomach cancer was conducted at four hospitals in Nagano prefecture, Japan, between October 1998 and March 2002. One hundred and fifty-three incident cases (in patients aged 20 to 74 years), of gastric cancer with histologic confirmation were newly diagnosed at these hospitals. For the 153 gastric cancer cases identified, two controls matched by age (within 3 years), sex, and residence area were randomly selected from the participants of a health check-up program during the same period in the same hospitals; they were confirmed to be cancer-free and with no past history of cancer. We conducted a questionnaire survey and obtained blood samples, with signed informed consent, from the 153 cancer-case patients and the 301 control subjects, before treatment for gastric cancer. Of the 153 gastric cancer cases, cardia cancer cases (in the upper third around the cardia), cases with uninformative anatomical subsite, and their control subjects were excluded from this study. A total of 126 non-cardia gastric cancer cases (middle body and antrum) and 247 controls remained. We also excluded subjects with extreme caloric intake (for men, <500 or ≥4000 kcal/day; for women, <400 or ≥3500 kcal/day), because this information was unreliable. Consequently, 122 non-cardia cancer cases and 235 controls were available for this analysis.

Questionnaire

The questionnaire was composed of items such as general characteristics (age, sex, sociodemographic characteristics), personal medical history, family history, smoking and drinking history, supplement use, and dietary factors. We also asked whether they were members of the Japan Agricultural Cooperatives (JA), because JA members may have particular dietary habits. JA members, who were farmers, were more familiar with health check-up programs than non-members. Therefore, we performed a careful multivariate adjustment for confounding factors, including JA membership, in order to exclude the effects of these factors.

All subjects were asked about the average frequency of intake and portion size of 141 items in the year preceding the interview or before a change of dietary habit, if the change had occurred in the past year. If they had any symptoms, their habitual intake prior to the symptoms was elicited. Daily consumption of rice and miso soup was classified into nine categories (<1, 1, 2, 3, 4, 5, 6, 7–9, 10+ cups/day). The frequency of other food items was classified into nine categories (never, 1–3 times/month, 1–2, 3–4, or 5–6 times/week, almost once/day, 2–3, 4–6 or >7 times/day), and the portion size was classified into three categories (more than 1.5 portions, same as the usual portion size, or less than half a portion). The mean daily consumption of each food group was calculated by multiplying the frequency and portion size. The estimated intake calculated by the questionnaire had been validated against a 14- or 28-day dietary record in a prior study. The Spearman correlation coefficients for intake of total vegetables, total fruits, pickled vegetables, and sodium were 0.36, 0.61, 0.74, and 0.59 in males and 0.34, 0.50, 0.75, and 0.55 in females, respectively [18,19].

Laboratory data

The study subjects were tested for serum pepsinogen I (PG I) and pepsinogen II (PG II), and for IgG antibody to *H. pylori* (Hp-Ab) and CagA (CagA-Ab). These antibodies were measured with an enzyme-linked

immunosorbent assay (ELISA; Helico G; Porton-Cambridge, Oxford, UK) kit and CagA kit (RADIM, Rome, Italy). Equal to and more than ten units per milliliter (U/ml) were considered a positive test in both Hp-Ab and CagA-Ab. *H. pylori* infection was defined when one or both serum assays were positive. The serum PG I and PG II levels were measured by radioimmunometric assay kits (PG1/PG2 RIABEAD; Dainabot, Tokyo, Japan). Atrophic gastritis was diagnosed according to the criteria of a PG I level below 70ng/ml and a PG I/PG II ratio below 3.0. The prevalence of serologically diagnosed atrophic gastritis using these criteria was well correlated ($r = 0.999$; $P < 0.0001$) with the age-adjusted mortality rate of gastric cancer among five Japanese populations [20].

To estimate the association between environmental factors and non-cardia cancer, the total intake of each food group and the frequency of each food item were each divided into three categories, at the nearest tertile based on the distribution in the control group. Smoking status was classified as never, past, or current smoker. The linear trend was assessed by assigning ordinal values for categorical factors.

In order to estimate the joint effects of environmental factors and *H. pylori* infection, the total consumption of vegetables, fruits, and salt was classified into a high-intake (above median intake) or a low-intake group (below median intake) according to the distribution of the control group. The number of cups per day was classified in two categories (high and low intake) for miso soup and rice. Smoking status was classified into two groups: never smoker and ever (past or current) smoker. We assessed the association with non-cardia cancer by taking into consideration the patterns of joint occurrence of both factors (environmental factors and *H. pylori* infection). Furthermore, the effect of interaction was checked by calculating an interaction term, and multiplying a dummy variable for each environmental factor by one for *H. pylori* infection.

Odds ratios (ORs) and 95% confidence intervals (95% CIs) and trends were obtained by conditional logistic regression analysis. All P values were two-sided and all the statistical analysis was performed using the SAS statistical software package [21].

Results

The mean ages of cases and controls were 57.8 and 57.4 years, respectively. The proportion of male participants was 67.2% in cases and 67.7% in controls.

Table 1 shows the crude and adjusted ORs of *H. pylori* combined with CagA for non-cardia cancer. The crude OR of *H. pylori* infection for non-cardia cancer was 7.0 (95% CI, 3.3–14.8). The highest OR was observed in the *H. pylori* seropositivity and CagA seropositivity [Hp(+)CagA(+)] category (OR, 10.1; 95% CI, 4.4–23.1). The *H. pylori* seropositivity and CagA seronegativity [Hp(+)CagA(−)] category had an estimated OR of 2.4, but this was not statistically significant. After adjustment for confounding variables, the ORs of *H. pylori* and/or CagA with gastric cancer increased.

Table 2 shows the association of environmental factors with the risk of non-cardia cancer. As for smoking, crude ORs for past and current smoking were 3.3 (95% CI, 1.6–6.7) and 2.8 (95% CI, 1.3–5.8), respectively. Past and current smoking remained, in the multivariate analysis, significantly associated with non-cardia cancer. Regarding salt intake, we found a slightly elevated OR (for high-intake category, OR, 1.5; 95% CI, 0.6–3.7), which did not reach statistical significance. As the consumption of miso soup increased, we found a significantly increased OR (for high-intake category, OR, 2.1; 95% CI, 0.9–5.1; P for trend = 0.04). A marginal positive association between rice intake and non-cardia cancer was observed (for the high-intake category, OR, 2.5; 95% CI, 1.0–6.1; P for trend = 0.07).

Table 1. Crude and adjusted odds ratios (ORs) and 95% confidence interval (CIs)[a] of *Helicobacter pylori* infection for non-cardia cancer

	Cases (n = 122)	Controls (n = 235)	Crude OR	95% CI	Adjusted OR[b]	95% CI
H. pylori infection combined with CagA						
H. pylori (−) and CagA (−)	10	84	1.0		1.0	
H. pylori (+) or CagA (+)	112	151	7.0	3.3–14.8	8.2	3.7–18.2
H. pylori (−) and CagA (+)	9	17	5.3	1.7–16.0	6.0	1.8–19.8
H. pylori (+) and CagA (−)	8	27	2.4	0.8–6.9	2.5	0.8–7.4
H. pylori (+) and CagA (+)	95	107	10.1	4.4–23.1	13.4	5.4–33.3

H. pylori infection was defined when one or both serum assays (*H. pylori* and CagA) were positive
JA, Japan Agricultural Cooperatives
[a] Conditional logistic regression analysis
[b] Adjusted for smoking status, JA membership, family history of gastric cancer, total vegetable intake, total fruits intake, and salt intake

Table 2. Crude and adjusted odds ratios (ORs) and 95% confidence intervals (CIs)[a] of environmental factors for non-cardia cancer

	Cases (n = 122)	Percentage	Controls (n = 235)	Percentage	Crude OR	95% CI	Adjusted OR	95% CI
Smoking[b]								
Never	44	(36.1)	119	(50.6)	1.0		1.0	
Past	39	(32.0)	53	(22.6)	3.3	1.6–6.7	2.8	1.3–6.3
Current	39	(32.0)	63	(26.8)	2.8	1.3–5.8	2.8	1.2–6.5
						0.01		0.03
Salt[c]								
Tertile 1 (8.7)	46	(37.7)	78	(33.2)	1.0		1.0	
Tertile 2 (13.0)	33	(27.0)	78	(33.2)	0.8	0.5–1.3	1.3	0.7–2.7
Tertile 3 (20.4)	43	(35.2)	79	(33.6)	0.9	0.6–1.5	1.5	0.6–3.7
						0.73		0.36
Miso soup[d]								
<3 cups/day	47	(38.5)	100	(42.6)	1.0		1.0	
3 cups/day	54	(44.3)	99	(42.1)	1.2	0.7–1.9	1.8	1.0–3.3
≧4 cups/day	21	(17.2)	36	(15.3)	1.2	0.6–2.4	2.1	0.9–5.1
						0.48		0.04
Pickled vegetables[d]								
Tertile 1 (10.9)	52	(42.6)	79	(33.6)	1.0		1.0	
Tertile 2 (36.6)	33	(27.0)	78	(33.2)	0.7	0.4–1.1	0.6	0.3–1.2
Tertile 3 (79.8)	37	(30.3)	78	(33.2)	0.7	0.4–1.2	0.6	0.3–1.3
						0.22		0.17
Rice[d]								
<4 cups/day	31	(25.4)	62	(26.4)	1.0		1.0	
4 cups/day	64	(52.5)	129	(54.9)	1.1	0.6–1.8	1.2	0.6–2.3
≧5 cups/day	27	(22.1)	44	(18.7)	1.4	0.7–2.8	2.5	1.0–6.1
						0.39		0.07
Total vegetable[e]								
Tertile 1 (119.2)	50	(41.0)	79	(33.6)	1.0		1.0	
Tertile 2 (218.4)	33	(27.0)	78	(33.2)	0.7	0.4–1.2	0.7	0.3–1.4
Tertile 3 (377.0)	39	(32.0)	78	(33.2)	0.8	0.5–1.3	0.9	0.4–2.2
						0.34		0.83
Total fruit[f]								
Tertile 1 (50.7)	44	(36.1)	77	(32.8)	1.0		1.0	
Tertile 2 (142.5)	43	(35.2)	80	(34.0)	1.0	0.6–1.6	1.4	0.7–2.6
Tertile 3 (294.0)	35	(28.7)	78	(33.2)	0.8	0.4–1.3	1.1	0.5–2.4
						0.36		0.73

Tertile 1, lowest tertile; tertile 2, intermediate tertile; tertile 3, highest tertile (median intake)
[a] Conditional logistic regression analysis
[b] Adjusted for *H. pylori* infection, JA membership, family history of gastric cancer, total vegetable intake, total fruit intake, and salt intake
[c] Adjusted for *H. pylori* infection, smoking status, JA membership, family history of gastric cancer, total vegetable intake, total fruit intake, and total energy intake
[d] Adjusted for *H. pylori* infection, smoking status, JA membership, family history of gastric cancer, total vegetable intake, total fruit intake, salt intake, and total energy intake
[e] Adjusted for *H. pylori* infection, smoking status, JA membership, family history of gastric cancer, total fruit intake, salt intake, and total energy intake
[f] Adjusted for *H. pylori* infection, smoking status, JA membership, family history of gastric cancer, total vegetable intake, salt intake, and total energy intake

High intake of pickled vegetables and low intake of total vegetables and fruits was not associated with non-cardia cancer.

The joint effects of environmental factors (smoking and foods) and *H. pylori* infection on the risk for non-cardia cancer are presented in Table 3. As for smoking status and *H. pylori* infection, the adjusted OR for non-cardia cancer was 1.9 (95% CI, 0.4–8.8) for the *H. pylori*-negative smoker group, 6.4 (95% CI, 2.1–19.7) for the *H. pylori*-positive never smoker group, and 19.0 (95% CI, 5.4–67.2) for the *H. pylori*-positive smoker group. An *H. pylori*-positive smoker had 3.0 (95% CI, 1.4–6.6) times the risk of non-cardia cancer compared to an *H. pylori*-positive never-smoker (data not shown in Table 3).

For salt, miso soup, pickled vegetables and rice, the ORs were compared with the *H. pylori*-negative subjects with lower intake as a reference. As for salt intake and *H. pylori* infection, the adjusted OR for gastric cancer was 1.8 (95% CI, 0.4–7.7) for *H. pylori*-negative

Table 3. Joint effect of environmental factors and *H. pylori* infection on the risk for non-cardia cancer

	Cases (n = 122)	Controls (n = 235)	Crude OR	95% CI	Adjusted OR	95% CI	Interaction term
Smoking[a]							
H. pylori (−) and never smoking	4	43	1.0		1.0		
H. pylori (−) and smoking	6	41	2.0	0.5–9.1	1.9	0.4–8.8	
H. pylori (+) and never smoking	40	76	5.3	1.8–15.8	6.4	2.1–19.7	
H. pylori (+) and smoking	72	75	17.7	5.2–60.5	19.0	5.4–67.2	0.52
Salt[b]							
H. pylori (−) and low intake	4	41	1.0		1.0		
H. pylori (−) and high intake	6	43	1.5	0.4–5.9	1.8	0.4–7.7	
H. pylori (+) and low intake	58	76	8.9	2.8–27.6	9.7	3.0–31.7	
H. pylori (+) and high intake	54	74	9.0	2.8–28.3	14.2	3.9–52.3	0.56
Miso soup[c]							
H. pylori (−) and low intake	8	77	1.0		1.0		
H. pylori (−) and high intake	2	7	2.1	0.3–13.7	3.0	0.4–24.1	
H. pylori (+) and low intake	93	122	7.6	3.4–17.0	9.0	3.8–21.3	
H. pylori (+) and high intake	19	29	8.4	3.0–23.4	12.6	4.0–39.4	0.52
Pickled vegetables[c]							
H. pylori (−) and low intake	6	45	1.0		1.0		
H. pylori (−) and high intake	4	39	0.9	0.2–3.5	0.7	0.2–3.3	
H. pylori (+) and low intake	63	73	7.4	2.7–20.2	8.0	2.8–22.8	
H. pylori (+) and high intake	49	78	5.7	2.1–15.7	6.4	2.0–19.9	0.84
Rice[c]							
H. pylori (−) and low intake	8	63	1.0		1.0		
H. pylori (−) and high intake	2	21	0.7	0.1–3.9	0.9	0.2–5.4	
H. pylori (+) and low intake	87	128	5.8	2.5–13.5	7.3	2.9–18.4	
H. pylori (+) and high intake	25	23	11.0	3.9–30.9	18.7	5.6–62.6	0.31
Total vegetable[d]							
H. pylori (−) and high intake	4	36	1.0		1.0		
H. pylori (−) and low intake	6	48	0.9	0.2–3.6	1.0	0.2–4.4	
H. pylori (+) and high intake	48	81	5.5	1.8–16.9	7.6	2.3–25.2	
H. pylori (+) and low intake	64	70	7.5	2.5–22.3	8.5	2.4–29.9	0.60
Total fruit[e]							
H. pylori (−) and high intake	5	39	1.0		1.0		
H. pylori (−) and low intake	5	45	0.8	0.2–3.2	0.9	0.2–3.9	
H. pylori (+) and high intake	44	78	4.8	1.7–13.0	5.8	2.0–16.9	
H. pylori (+) and low intake	68	73	7.9	2.8–21.8	10.6	3.3–33.9	0.32

Salt, pickled vegetable, total vegetable and fruit ; low (below median) intake , high (above median) intake
Miso soup; low intake (<3 cups/day), high intake (≧3 cups/day)
Rice; low intake (<4 cups/day), high intake (≧4 cups/day)
H. pylori infection was defined when one or both serum assays (*H. pylori* and CagA) were positive
[a] Adjusted for JA membership, family history of gastric cancer, total vegetable intake, total fruit intake, and salt intake
[b] Adjusted for JA membership, smoking status, family history of gastric cancer, total vegetable intake, total fruit intake, and total energy intake
[c] Adjusted for JA membership, smoking status, family history of gastric cancer, total vegetable intake, total fruit intake, salt intake and total energy intake
[d] Adjusted for JA membership, smoking status, family history of gastric cancer, total fruit intake, salt intake, and total energy intake
[e] Adjusted for JA membership, smoking status, family history of gastric cancer, total vegetable intake, salt intake, and total energy intake

subjects with a high intake, 9.7 (95% CI, 3.0–31.7) for *H. pylori*-positive subjects with a low intake, and 14.2 (95% CI, 3.9–52.3) for *H. pylori*-positive subjects with a high intake. After adjustment for confounding factors including smoking status, high intake of miso soup tended to increase the ORs of non-cardia cancer in both *H. pylori*-positive and -negative subjects. Regarding rice consumption, the adjusted OR was 7.3 (95% CI, 2.9–18.4) for *H. pylori*-positive subjects with a low intake and 18.7 (95% CI, 5.6–62.6) for *H. pylori*-positive subjects with a high intake. High rice consumption was not associated with non-cardia cancer among *H. pylori*-negative subjects.

ORs were compared with *H. pylori*-negative subjects with a high intake of total vegetables and total fruits as the reference. Low intake of vegetables/fruits did not increase the risk compared with high intake among *H. pylori*-negative subjects. However, a marginal positive association between low intake of total fruits and non-cardia cancer was observed among *H. pylori*-positive subjects.

Additionally, no significant improvement in the regression model was observed when the interaction term between these environmental factors (smoking, food groups, and food items) and *H. pylori* infection was added.

Discussion

In this study, *H. pylori* infection was defined as present when one or both serum assays were positive. Clinical data indicated that the CagA antibody persisted longer after eradication treatment than the antibody detected by *H. pylori* IgG [22]. It seems reasonable to assume that the addition of a test for the CagA antibody will result in a more correct representation of past exposure than the use of the *H. pylori* IgG antibody alone, as Ekstrom et al. [23] suggested.

We confirmed the strong association between *H. pylori* infection and non-cardia cancer. In this study, a significantly increased risk was observed in the *H. pylori* seropositivity and CagA seropositivity [Hp(+)CagA(+)] category, while risk was moderately but not significantly increased between the *H. pylori* seropositivity and CagA seronegativity category. This suggested that CagA-positive *H. pylori* infection had strongly increased the risk for non-cardia cancer compared with *H. pylori*-negative subjects, consistent with previous studies [3–6].

As for smoking status, smoking tended to increase the risk for gastric cancer irrespective of *H. pylori* infection. *H. pylori*-positive smokers showed the highest OR, of 19.0, compared to *H. pylori*-negative never-smokers. Smoking proved to be a risk factor even among subjects with *H. pylori* infection. Some mechanisms of smoking associated with the increased risk of gastric cancer have been suggested. Smokers show lower plasma levels of antioxidants such as vitamin C and beta-carotene [24], and tobacco smoke contains carcinogenic nitrosamines, triggering the carcinogenesis of gastric carcinoma [25]. An increased risk of gastric cancer from smoking was observed in two prospective studies [26,27]. From investigating the joint effects of *H. pylori* and smoking status, Siman et al. [28] suggested that smoking and *H. pylori* were both risk factors for gastric cancer, and that smoking was still a risk factor among *H. pylori*-positive individuals. Brenner et al. [16] found that CagA-positive smokers had an increased risk of non-cardia gastric cancer in a close to multiplicative way, leading to a 16.6-fold risk increase compared with non-smokers without *H. pylori* infection. In a Russian case-control study, smoking had no effect on the risk of gastric cancer in *H. pylori*-negative men, but it was associated with a significantly increased risk in *H. pylori*-positive men (*P* value for interaction, 0.07) [29].

Excessive salt intake is a risk factor supported by experimental and epidemiological studies [10,30,31]. The promoting effects of salt may be caused through a mechanism by which high salt concentrations destroy the mucosal barrier that protects the surface membrane of the stomach [32]. In Mongolian gerbils, the synergistic promoting effects of *H. pylori* infection and a high-salt diet (containing 10% sodium chloride) on gastric carcinogenesis were observed [31]. In the present study, although high salt intake caused a slightly increased OR, it was not statistically significant. In the analysis of joint effects, *H. pylori* infection with a high salt intake had an elevated OR for non-cardia cancer compared with the reference category, but failed to show a statistically significant increase in ORs compared with *H. pylori*-positive subjects with a low intake (data not shown). Part of the reason for this result was the difficulty in estimating the exact amount of salt consumption from the questionnaire. Moreover, we may find a synergistic promoting effect of salt intake on gastric cancer only in subjects who consistently consume high levels of salt.

As miso soup and rice intake increased, we found a steady increase in the risk of non-cardia cancer. Because miso soup is one of the major contributors to sodium intake, this result is plausible. For rice, a marginal positive association was observed. A high rice intake significantly increased the risk (OR, 2.6; 95% CI, 1.1–6.0) compared to low intake even in *H. pylori*-positive subjects. A positive association between high rice intake and *H. pylori* infection has been observed in some epidemiological studies [8,9]. Although a previous study suggested that carbohydrates irritated the gastric mucosa [33], the direct effect of rice on carcinogenesis in the stomach was unclear. As an indirect effect, there is a possibility that rice intake is a marker of salt, salty food, and other dietary factors that are risk factors for gastric cancer.

Vegetables and fruits contain many compounds with anticarcinogenic properties, including carotenoids and vitamin C, and have been considered to be factors that protect against stomach carcinogenesis [10,11]. Our previous study showed the protective effect of mushrooms and cruciferous vegetables on the risk of gastric cancer among the same subjects as those in this present study [12]. The high consumption of total vegetables and fruits did not decrease the risk of non-cardia cancer after adjustment for *H. pylori* infection in our study. In the analysis of *H. pylori* infection and vegetables/fruits, low intake did not show an increased risk for non-cardia cancer compared with *H. pylori*-positive subjects with high intake, although *H. pylori*-positive subjects with low intake of total fruits tended to have an increased risk compared with *H. pylori*-positive subjects with high intake. In a previous study using the same food frequency questionnaire (FFQ), the average intake of vegetables and fruits was 215 g/day and 132 g/day among 18399 men, and 256 g/day and 202 g/day among 20932 women [34]. The lack of association could also be explained by a relatively high intake of these items in the two municipalities of Nagano prefecture comprising the present study area compared with that in other areas

[35]. Kobayashi et al. [36], in a prospective study, suggested that vegetable and fruit intake, even in low amounts, was associated with a decreased risk; however, the risk did not decrease in a stepwise manner as the consumption increased. Therefore the decreased risk may have not been observed in the present study because these study subjects consumed relatively high amounts of vegetables and fruits. Another explanation may be that the protective effect of vegetable consumption, if any, may be modified or masked by other prevalent risk factors, for example, *H. pylori* infection.

The Spearman correlation coefficient for total vegetable intake assessed with FFQ and 14- or 28-day dietary records was relatively low. However, the intake assessed with FFQ was comparable to that with the 14- or 28 days dietary record in the categorization of vegetable intake [18]. Moreover, we had observed an inverse association between vegetable intake and the risk of colorectal cancer in a simultaneously conducted case-control study using the same FFQ [12]. It is, therefore, unlikely that the failure to observe a protective association was due to the relatively low validity of the vegetable intake assessment.

In this study, there was almost no improvement at all in the logistic regression model when the interaction term between these environmental factors and *H. pylori* infection was added. The limited number of cases prohibited a more comprehensive assessment of gastric cancer risk according to the joint classification of subjects by environmental factors and *H. pylori* infection, and led to quite wide confidence intervals for some of the risk estimates. In other words, we might have failed to detect a statistically significant interaction owing to the small number of *H. pylori-negative* cases. Moreover, the high prevalence of *H. pylori* infection and its strong link to gastric cancer may have made precise evaluation of the association between environmental factors and gastric cancer virtually impossible.

There were some limitations in this study. First, the dietary information we collected was about the diet 1 year before the diagnosis. Such dietary information may not actually reflect past dietary habits, which may be more important for carcinogenesis in the stomach. Additionally, one of the major problems of case-control studies is the possibility of recall bias due to knowledge of the disease status. Information on food intake was collected after subsequent diagnosis of gastric cancer, so we could not avoid the recall bias inherent in case-control studies. Furthermore, cases might have changed their dietary habits as a consequence of gastric cancer and atrophic gastritis. Thus, dietary assessment is subject to considerable misclassification. In addition, the diagnosis of *H. pylori* infection (including CagA) was based on immunological tests. The possibility of misclassification of *H. pylori* and CagA seropositivity by immunological tests was not excluded. However, if the misclassification occurred equally among all subjects, the risk of infection linked to the development of gastric cancer was underestimated. Another limitation of case-control studies is their potential selection bias. Among the controls in this study, the percentage of never-smokers was high because of the low number of female smokers. The percentages of past smokers and current smokers were 31.5% and 39.0%, respectively, in the male controls. The percentage of current male smokers in this study was low compared with that in a population-based prospective study of 19576 Japanese men aged 40–59 years (53.4%) [26]. The low prevalence of smokers in the control group may have led to an overestimation of OR regarding cigarette smoking. Because the controls were participants in a medical health check-up program, they were considered to be more health-conscious than the general population. In addition, the JA members were more familiar with health check-up programs than non-members. Thus, we performed careful multivariate adjustment for confounding factors, including JA membership, as mentioned above.

We confirmed the strong association of *H. pylori* combined with CagA with non-cardia cancer. Regarding environmental factors, smoking and high intake of miso soup were associated with non-cardia cancer, regardless of *H. pylori* infection. Although a high rice intake and low fruit intake increased risk only among *H. pylori*-positive subjects, the interaction term was not statistically significant. However, we could not deny the interaction between *H. pylori* infection and environmental factors. Among the various factors studied here, *H. pylori* infection was shown to be the most important factor for non-cardia gastric cancer, indicating that eradication therapy for *H. pylori* would possibly be an effective strategy for reducing the risk of gastric cancer. Our study also indicates that other realistic alternatives may be smoking cessation and dietary modification for non-cardia gastric cancer, irrespective of *H. pylori* status. Further studies in a large number of subjects will be necessary to clarify the interaction between the various risk factors.

Acknowldgments The authors gratefully acknowledge the generous assistance of the staff members of each hospital and the Agricultural Technology Institute of the Nagano Farmers' Fenderation, Ms. Aoki, Mr. Ueki, Ms. Kimijima, Ms. Komatsu, Mr. Shimazaki, Ms. Horano, Mr. Yajima, Dr. Ikegawa, and Dr. Matsuzawa, and the laboratory staff member, Ms. Hashimoto. This study was supported in part by the Agricultural Technology Institute of the Nagano Farmers' Federation, a Grant-in-Aid for Cancer Research and the Second Term Comprehensive 10-year Strategy for Cancer Control from the Ministry of Health and Welfare of Japan,

and a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology, Japan.

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British Journal of Cancer (2004) 90, 128–134

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Salt and salted food intake and subsequent risk of gastric cancer among middle-aged Japanese men and women

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Evidence on the association between salt intake and gastric cancer is sparse, especially in prospective studies. We conducted a population-based prospective study in Japan, where the majority of men has been infected with *Helicobacter pylori*. A total of 18 684 men and 20 381 women aged 40–59 years who reported their dietary habits and did not report any serious disease at baseline were followed from 1990 to 2001. A total of 486 cases, 358 men and 128 women, with histologically confirmed gastric cancer were documented among them. The quintile category of salt intake was dose-dependently associated with gastric cancer risk in men after adjusting for potential confounding factors (P for trend <0.001), while a trend was not clear in women (P for trend $=0.48$). Although stratification by study area, with varied salt intake and gastric cancer incidence, attenuated the observed clear associations with salt and salted foods, the frequency categories of highly salted foods such as salted fish roe and salted fish preserves were strongly associated with the risk in both sexes. Restriction of salt and salted food intake is a practical strategy to prevent gastric cancer in areas with high risk.

British Journal of Cancer (2004) **90,** 128–134. doi:10.1038/sj.bjc.6601511 www.bjcancer.com

Keywords: diet; prospective studies; salts; stomach neoplasms

Gastric cancer is the second most frequent cause of cancer deaths worldwide, with an estimated 776 000 deaths in 1996, almost two-thirds of which were in developing countries (World Health Organization, 1997). In Japan, gastric cancer killed 50 000 people in 2000, which was the second most frequent cancer death (17% of all cancer deaths; Ministry of Health, Labor and Welfare, Japan). Moreover, a total of 100 000 new cases of gastric cancer were estimated in Japan in 1996, and it was the most common cancer site (21% of all incident cancers; Research Group for Population-based Cancer Registration in Japan, 2002). Therefore, prevention of gastric cancer is one of the most important elements in any cancer control strategy both in Japan and around the world.

Although salt and salted foods are probable risk factors, based on evidence from a large number of case–control and ecological studies (Tsugane *et al*, 1992b; Joossens *et al*, 1996; Kono and Hirohata, 1996), evidence from prospective investigation is scarce and inconsistent (Nomura *et al*, 1990; Kneller *et al* 1991; Kato *et al*, 1992). The recent report of a joint WHO/FAO Expert Consultation concluded that 'Salt-preserved foods and salt probably increase the risk of stomach cancer' (World Health Organization, 2003). The difficulties in estimating the intake of salt *per se* may account for divergent findings.

We tested this hypothesis in Japan in a population-based prospective study in four public health centre areas with varying gastric cancer mortality rates and where the majority (75%) of randomly selected men aged 40–49 years has been infected with *Helicobacter pylori* (Tsugane *et al*, 1994).

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Received 26 August 2003; revised 10 October 2003; accepted 15 October 2003

MATERIALS AND METHODS

Study cohort

The Japan Public Health Center-based prospective study on cancer and cardiovascular diseases (JPHC Study) Cohort I, which was partly reported elsewhere (Tsugane *et al*, 1999), provided a basis for this investigation. As of 1 January 1990, we established a population-based cohort of 54 498 people (27 062 men and 27 436 women) who resided in 14 administrative districts supervised by four regional public health centres (PHCs): 12 291 from Ninohe City and Karumai Town in the Ninohe PHC area of Iwate prefecture, 15 782 from Yokote City and Omonogawa Town in the Yokote PHC area of Akita, 12 219 from eight districts of Minami-Saku county in the Saku PHC area of Nagano and 14 206 from Gushikawa City and Onna Village in the Ishikawa PHC area of Okinawa. All were born between 1930 and 1949 (40–59 years of age at the baseline). These PHC areas were selected to represent the extent of variation in the mortality rate of stomach cancer based upon our previous ecological study (Tsugane *et al*, 1992a; Tsubono *et al*, 1997). The institutional review board of the National Cancer Center approved this study.

Baseline survey

In 1990, a self-administered questionnaire was distributed to all registered residents. They were asked to report on their socio-

demographics, personal medical history, smoking and drinking history and diet. A total of 43 149 subjects, 20 665 men (76%) and 22 484 women (82%), returned their questionnaires. Although the date of questionnaire completion ranged from January 1990 to May 1992, 54% responded between February 1990 and March 1990. Only 4% were completed after October 1990.

The weekly intake frequency of 27 food items was reported in four categories: rarely, 1–2 days week^{-1}, 3–4 days week^{-1} and almost daily (5+ days week^{-1}). For rice, miso soup and nine kinds of beverage, the daily amount consumed was also reported. Design and participation rate of the baseline survey and dietary habits among JPHC study participants at baseline survey were reported elsewhere (Tsugane and Sobue, 2001; Tsugane *et al*, 2001).

Based on dietary record data, we developed a food composition table that corresponded to the food items listed in the questionnaire and determined standard portions/units for each food category using the observed median values. The food composition table was developed and standard portions/units were assigned separately for men and women. Daily nutrient intakes were calculated by multiplying the consumption frequency of each food by the nutrient content of the standard portions/units and summing these values for all foods. Calculation of total energy and 30 nutrients was done with the Standard Tables of Food Composition (Science and Technology Agency, 1982) and other sources. The validity was assessed among subsamples (94 men and 107 women) with actual nutrient intake, which was calculated based on a 28-day dietary record (7 consecutive days in four seasons; 14 days in the Ishikawa PHC area; Tsugane *et al*, 2003). The Spearman rank correlations between two indices for sodium intake were 0.49 in men and 0.54 in women (Tsubono *et al*, 2003). Those with two-time 24-h urine excretion level (32 men and 57 women, except Ishikawa PHC area) were 0.38 in men and 0.12 in women (Tsubono *et al*, 2003).

The following food items were considered as salted food in our analysis: miso soup (salt content: 0.5–1.2%), pickled vegetables (1–10%; specific foods include shiozuke, with 1.7% in Chinese cabbage, 2.8% in cucumber, and nuka-misozuke, with 1.7% in eggplant, 2.8% in cucumber), salted fish roe (tarako, salted Alaska pollack roe, 6.6%, suziko or ikura, salted salmon roe, 9.7%), salted fish preserves (shiokara, salted fish guts, 11.4% in squid, neri-uni, salted gonad paste of sea urchin, 11.9%) and dried or salted fish (mezashi, salted and semidried skewered sardine, 4%, and shio-sake, salted salmon, 8%). Salt content data for specific food items were taken from with Standard Tables of Food Composition (Science and Technology Agency, 1982). The Spearman rank correlations with the dietary records (g day^{-1}) were 0.41 for miso soup, 0.67 for pickled vegetables, 0.61 for salted fish roe, 0.43 for salted fish preserves and 0.55 for dried or salted food in men, and 0.49, 0.61, 0.47, 0.34 and 0.33 in women, respectively (Tsugane *et al*, 2003). Miso soup was categorised into the following four groups in this analysis: not daily, 1 cup day^{-1}, 2 cups day^{-1}, 3+ cups day^{-1}.

We excluded subjects with a self-reported serious illness (cancer, cerebrovascular disease, myocardial infarction, or chronic liver disease) at baseline as well as non-Japanese subjects and subjects who had already moved away at the baseline, which we confirmed during the follow-up period. We further excluded subjects who reported extreme total energy intake (upper 2.5% or lower 2.5%). These exclusions left 18 684 eligible men and 20 381 women included in this study.

Follow-up and identification of gastric cancer

Death and change in residence We followed all registered cohort subjects from 1 January 1990 to 31 December 2001. In Japan, all death certificates are submitted to a local government office and forwarded to the PHC in the area of residence. Mortality data are then sent to the Ministry of Health and Welfare and coded for inclusion in the National Vital Statistics. The registration of deaths in Japan is required by the Family Registration Law and is believed to be complete. Therefore, all deaths of cohort subjects were based upon death certificates from each PHC, whenever they stayed in their original area. The changes in residence status were identified annually through the residential registry in each area.

Cancer registry for JPHC study Newly diagnosed cases of cancer were reported by hospitals in and around the study areas when the birth date and residence fulfilled cohort inclusion criteria. Candidate patients were linked by name, address, and date of birth and entered in the cancer registry for the JPHC Study Cohort I. In the Ninohe and Ishikawa PHC areas, a prefecture-wide cancer registry was available (i.e. Iwate and Okinawa Prefecture cancer registry, respectively). Death certificates were used as a supplementary information source for the cancer registry, and 259 cases were first notified by it. As of July 2002, such cases accounted for 7.6% (DCN (Death Certificate Notification) %) of all the 3429 entries, which had been diagnosed in 1990–2001. Among them, 39 cases were not confirmed by medical records, and they would account for 1.1% (DCO (Death Certificate Only) %) of all the entries.

Identification of gastric cancer Cases of gastric cancer were extracted from the cancer registry for the JPHC Study, based on site (International Classification of Diseases for Oncology (ICD-O) code: C160–169) and histological confirmation by biopsy or surgery. A total of 486 cases of gastric cancer, 358 in 18 684 men and 128 in 20 381 women, were documented as of July 2002 with a histologically proven diagnosis in 1990–2001. This left 10 cases without histological confirmation, which we considered not diagnosed with gastric cancer.

Cardia cancer was defined as a tumour located in the oesophagogastric junction or upper third of the stomach (International Classification of Diseases for Oncology (ICD-O) code C160–161; World Health Organization 1990). A tumour located in the lower side of the stomach was classified as distal gastric cancer (ICD-O code C162–167). Those subsites that could not be classified for its diffuse lesion (ICD-O code C168) or those with no information (ICD-O code C169) were categorised as unclassified. Histological subdivisions were made according to Lauren's (1965) classification.

Statistical analysis

Person-years at risk were calculated from 1 January 1990 to the date of diagnosed gastric cancer, the date of death, or change in residence or 31 December 2001, whichever came first. A Cox's proportional-hazards model was used to calculate the relative risk (RR) by category of each frequency of dietary intake at baseline after adjusting for covariates using PROC PHREG of the SAS program (SAS Institute, Inc., Cary, NC, USA). Cigarette smoking, fruit and non-green–yellow vegetable intake and age were principally adjusted based on our previous findings (Kobayashi *et al*, 2002; Sasazuki *et al*, 2002). Considering the observed colinearity of the intake frequency of three kinds of vegetables (e.g. green, yellow and other vegetables), only non-green–yellow vegetable intake was included in the multivariate model, because it was more clearly associated with gastric cancer risk (Kobayashi *et al*, 2002). Although analyses by histological type and by anatomical subsite were conducted, the results are not shown because of unstable findings due to reduced power.

RESULTS

Age-adjusted incidence rates per 100 000 person-years with number of subjects, person-years of follow-up and number of gastric cancer cases are shown by sex and study area in Table 1, as well as the proportion of each anatomic subsite and histological type. Those rates were three times higher in the men of all three PHC areas except Ishikawa, with similar rates, and differed substantially by PHC area, especially among men. There were six- and two-fold differences with regard to study area in men and women, respectively. Most tumours (70% in men, 75% in women) were located in the distal portion, and there were no significant differences according to sex or study area. As to histological type, the differentiated type of gastric cancer was predominant in men (61%), whereas it was less common in women (37%). Among men, the proportion of differentiated type was slightly higher in the Saku PHC area and lower in Ishikawa PHC area.

The baseline characteristics according to each quintile category of salt intake are shown in Table 2. Approximately 60% of Ishikawa subjects were categorised in the lowest quintile, whereas the majority of Ninohe and Yokote subjects were located in the upper two quintiles. Smokers were more common in upper quintiles of salt intake in men, but they were more common in lower quintiles in women. The intake frequencies of five salted foods were closely correlated with salt intake. The frequencies of fruit and three kinds of vegetable intake were also positively correlated with salt intake, although not remarkable.

The Relative risks and 95% confidence interval (CI) of each quintile category of salt intakes are shown in Table 3; these were calculated after adjusting for age, cigarette smoking and fruit and vegetable intake. The quintile category of salt intake was dose-

Table 1 Number of subjects and incident gastric cancer by sex and study area

Sex and study area	Number of subjects	Person-years of follow-up	Number of incident gastric cancers	Age-adjusted incidence rate per 100 000 person-years	Anatomic subsite cardia/distal/unknown	Histological type differentiated/ undifferentiated/ unclassified
Men	18 684	207 110	358	173	42/250/66	218/117/23
Ninohe, Iwate	3930	43 080	43	104	4/23/16	25/17/1
Yokote, Akita	5046	56 665	168	295	22/126/20	100/63/5
Saku, Nagano	5034	56 016	118	213	15/82/21	84/26/8
Ishikawa, Okinawa	4674	51 349	29	55	1/19/9	9/11/9
Women	20 381	232 463	128	55	12/96/20	47/71/10
Ninohe, Iwate	4602	52 484	18	35	2/10/6	9/9/0
Yokote, Akita	5858	67 506	57	84	5/46/6	23/31/3
Saku, Nagano	5135	58 912	36	61	4/26/6	11/22/03
Ishikawa, Okinawa	4786	54 061	17	31	1/14/2	4/9/4

Table 2 Background characteristics of subjects by quintiles of salt intake

	Men ($n = 18\,684$)					Women ($n = 20\,381$)				
Quintile	**1 (low)**	**2**	**3**	**4**	**5 (high)**	**1 (low)**	**2**	**3**	**4**	**5 (high)**
Median (g/d)[a]	**2.9**	**4.8**	**6.1**	**7.5**	**9.9**	**2.6**	**4.2**	**5.3**	**6.4**	**8.2**
Study area (%)										
Ninohe, Iwate	8	15	21	27	35	10	16	24	30	33
Yokote, Akita	13	23	29	33	37	15	24	31	34	39
Saku, Nagano	19	28	32	32	22	19	30	29	27	21
Ishikawa, Okinawa	60	33	17	8	6	56	29	16	9	6
Age (years)	48.7	49	49.4	49.9	50.1	48.9	49.3	49.7	50	50
Current smokers (%)	49	51	54	56	56	8.2	6.5	4.7	3.6	4.2
Daily alcohol drinkers (%)	25	34	42	46	47	2.1	2.7	1.8	2	2.2
Salted food intake										
Miso soup (bowls week^{-1})	4.1	8.3	12.2	15.2	20.6	3.5	6.8	10.8	13.8	17.3
Pickled vegetables (days week^{-1})	1.4	2.8	3.9	4.7	5.1	1.8	3.4	4.3	5	5.4
Salted fish roe (days week^{-1})	0.4	0.8	1.1	1.5	2.2	0.4	0.9	1.1	1.4	2.1
Salted fish preserves (days week^{-1})	0.2	0.5	0.8	1.1	1.7	0.2	0.4	0.5	0.7	1.2
Dried or salted fish (days week^{-1})	0.9	1.5	1.9	2.3	2.9	1.1	1.8	2.1	2.4	2.9
Fruit and vegetables										
Fruit (days week^{-1})	2.3	2.9	3.1	3.4	3.5	3.2	4	4.3	4.5	4.5
Green vegetables (days week^{-1})	3.2	3.4	3.6	3.6	4	3.6	3.9	4	4.1	4.2
Yellow vegetables (days week^{-1})	2.4	2.6	2.7	2.8	2.9	3.1	3.3	3.4	3.5	3.5
Other vegetables (days week^{-1})	3	3.5	3.9	4.1	4.4	3.5	4.1	4.4	4.6	4.7

[a]Based on food frequency questionnaire.

Table 3 Multivariate relative risks (RR) of incident gastric cancer by quintiles of salt intake at baseline in men and women: JPHC Study, 1990–2001

	Quintiles of salt intake					
	1 (low)	**2**	**3**	**4**	**5 (high)**	***P* for trend**
Men						
Person-years of follow-up	40 655	41 445	41 165	41 831	42 016	
No. of incident cancers	35	63	73	95	92	
Age-adjusted incidence rate	93	158	179	216	209	
Multivariate RR[a]	1	1.74	1.96	2.3	2.23	
95% CI	Reference	1.14–2.66	1.30–2.97	1.53–3.46	1.48–3.35	<0.001
Multivariate RR[b]	1.00	1.36	1.35	1.54	1.5	
95% CI	Reference	0.89–2.09	0.88–2.07	1.01–2.34	0.98–2.29	0.08
Women						
Person-years of follow-up	45 828	46 196	46 811	47 097	47 032	
No. of incident cancers	23	22	26	19	38	
Age-adjusted incidence rate	52	48	55	39	77	
Multivariate RR[a]	1	0.86	0.96	0.58	1.32	
95% CI	Reference	0.47–1.56	0.54–1.72	0.30–1.12	0.76–2.28	0.48
Multivariate RR[b]	1.00	0.75	0.81	0.48	1.09	
95% CI	Reference	0.41–1.37	0.45–1.48	0.25–0.95	0.61–1.94	0.85

[a]Adjusted for age in 1990 (40–44, 45–49, 50–54, 55–59 years), cigarette smoking (never, past, current) and fruit and non-green–yellow vegetable intake (almost none, 1–2 days week^{-1}, 3–4 days week^{-1}, almost everyday). [b]Further stratified by PHC area. CI = confidence interval.

dependently associated with gastric cancer risk in men (*P* for trend <0.001) and it was attenuated after stratifying by study area (*P* for trend = 0.08). Salt intake was not associated with gastric cancer risk in women.

RR and 95% CI of intake of five salted food categories are shown in Table 4 for men and Table 5 for women; these were calculated after adjusting for age, cigarette smoking and fruit and vegetable intake. The frequency categories of all listed salted foods were closely associated with the risk in men (*P* for trend <0.05). Although the observed associations were attenuated after further adjusting for salt intake or further stratifying by study area, they were still evident for salted fish roe (*P* for trend <0.001 after adjusting for salt intake and 0.08 after stratifying by study area) and salted fish preserves (*P* for trend <0.001 and 0.03, respectively). For women, there were no clear associations for intake of miso soup, pickled vegetables and dried or salted fish. However, the associations for salted fish roe and salted fish preserves were clear (*P* for trend = 0.003 for salted fish roe and 0.03 for salted fish preserves) and they were still evident even after further adjusting for salt intake (*P* for trend = 0.006 for salted fish roe and 0.06 for salted fish preserves). These associations were still persistent, although not statistically significant, even after stratifying by study area.

DISCUSSION

In this population-based prospective study of middle-aged Japanese men and women, we observed a dose-dependent increased risks of gastric cancer with the consumption of highly salted foods such as salted fish roe and salted fish preserves. In men, the associations were even clear for estimated total salt and the intake of other salted foods such as miso soup, pickled vegetables and dried or salted fish.

In experimental studies with rats, ingestion of salt is known to cause gastritis, and when coadministered, it enhances the carcinogenic effects of known gastric carcinogens such as *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (MNNG; Tatematsu *et al*, 1975; Takahashi and Hasegawa, 1985). Moreover, many epidemiological studies showed that salt or salted food intake increased the risk of gastric cancer (Kono and Hirohata, 1996), including those conducted among Japanese (Tajima and Tominaga, 1985; Hoshiyama and Sasaba, 1992; Tsugane *et al*, 1992b). Intragastric high-salt concentration destroys the mucosal barrier, and leads to inflammation and damage such as diffuse erosion and degeneration. The induced proliferous change may enhance the effect of food-derived carcinogens (Takahashi and Hasegawa, 1985). Such mucosal damage also enhances *H. pylori* colonisation in mice (Fox *et al*, 1999) and possibly in humans (Tsugane *et al*, 1994), thereby inducing chronic gastritis (Blaser, 1990) and possibly increasing the risk of gastric cancer (International Agency for Research on Cancer, 1994).

The geographic difference observed in this prospective study confirmed our previous findings from an ecological study conducted in the identical study areas in 1989–1990 (Tsugane *et al*, 1991). The male age-standardised mortality rate (world population) per 100 000 population for 1985–1987 were 29.7 in Ninohe, 49.4 in Yokote, 42.8 in Saku and 17.2 in Ishikawa, respectively. In that study, average amount of salt excretion in 24-h urine (g day^{-1}) from randomly selected men aged 40–49 years was closely associated ($r^2 = 0.995$) with the gastric cancer mortality in these areas: 9.9 in Ninohe, 13.4 in Yokote, 11.9 in Saku and 8.0 in Ishikawa. However, this strong correlation was largely attenuated ($r^2 = 0.265$) with average amounts of dietary salt intake (g day^{-1}) based on 3-day dietary record and calculated from the Standard Tables of Food Composition (Science and Technology Agency, 1982): 15.5 in Ninohe, 15.0 in Yokote, 13.2 in Saku and 12.0 in Ishikawa. The average amount of estimated salt intake (g day^{-1}) in this study with limited food items was based on the standard composition table (Science and Technology Agency, 1982): 7.7 in Ninohe, 7.3 in Yokote, 6.6 in Saku and 4.5 in Ishikawa. Therefore, the estimated salt intake was relatively overestimated in Ninohe and may have underestimated the true association between salt intake and gastric cancer incidence, especially in women. The precise estimation of salt intake may be implausible especially in different study areas, where the content of salt may be differed in each area even for the same food item. Although multiple 24-h urine collections may be an ideal method to estimate habitual salt intake (Liu *et al*, 1979), they are not feasible for a large-scale prospective study.

The observed weak link between salt intake and gastric cancer in women may also be due to relatively low validity of estimated salt intake (Tsubono *et al*, 2003). The Spearman rank correlation with 28-day dietary record was 0.54 in women, however, that with 2-day

Table 4 Relative risks (RR) of incident gastric cancer by category of salted food intake at baseline in men: JPHC Study, 1990–2001

	Category of intake				
	Not daily	**1 cup day^{-1}**	**2 cups day^{-1}**	**3 or more cups day^{-1}**	***P* for trend**
Miso soup					
Multivariate RR[a]	1	1.76	1.99	1.75	
95% CI	Reference	1.16–2.67	1.39–2.87	1.22–2.51	0.002
Multivariate RR[b]	1	1.51	1.37	1.01	
95% CI	Reference	0.98–2.32	0.90–2.10	0.64–1.61	0.29
Multivariate RR[c]	1	1.52	1.62	1.4	
95% CI	Reference	1.00–2.31	1.12–2.34	0.97–2.03	0.05
	Almost none	**1–2 days week^{-1}**	**3–4 days week^{-1}**	**Almost everyday**	
Pickled vegetables (e.g. shiozuke, nuka-misozuke)					
Multivariate RR[a]	1	1.54	2.71	2.35	
95% CI	Reference	0.97–2.46	1.76–4.19	1.57–3.54	<0.001
Multivariate RR[b]	1	1.4	2.28	1.86	
95% CI	Reference	0.87–2.24	1.45–3.59	1.19–2.89	0.008
Multivariate RR[c]	1	1.09	1.43	1.17	
95% CI	Reference	0.68–1.77	0.89–2.30	0.74–1.84	0.69
Salted fish roe (e.g. tarako, suziko, ikura)					
Multivariate RR[a]	1	1.58	2.18	2.44	
95% CI	Reference	1.23–2.04	1.60–2.97	1.41–4.23	<0.001
Multivariate RR[b]	1	1.42	1.94	2.21	
95% CI	Reference	1.09–1.85	1.39–2.70	1.24–3.92	<0.001
Multivariate RR[c]	1	1.02	1.29	1.45	
95% CI	Reference	0.78–1.34	0.92–1.80	0.83–2.56	0.08
Salted fish preserves (e.g. shiokara, neri-uni)					
Multivariate RR[a]	1	1.47	1.75	3.12	
95% CI	Reference	1.17–1.84	1.21–2.53	1.68–5.78	<0.001
Multivariate RR[b]	1	1.3	1.53	2.76	
95% CI	Reference	1.02–1.66	1.03–2.28	1.44–5.27	<0.001
Multivariate RR[c]	1	1.07	1.29	2.33	
95% CI	Reference	0.85–1.36	0.88–1.87	1.25–4.33	0.03
Dried or salted fish (e.g. mezashi, shio-sake)					
Multivariate RR[a]	1	2.16	1.85	2.23	
95% CI	Reference	1.53–3.05	1.26–2.70	1.37–3.63	0.007
Multivariate RR[b]	1	1.83	1.49	1.76	
95% CI	Reference	1.28–2.62	1.00–2.23	1.06–2.93	0.21
Multivariate RR[c]	1	1.15	0.94	1.2	
95% CI	Reference	0.79–1.70	0.62–1.43	0.71–2.02	0.74

[a]Adjusted for age in 1990 (40–44, 45–49, 50–54, 55–59 years), cigarette smoking (never, past, current) and fruit and non-green–yellow vegetable intake (almost none, 1–2 days $^{-1}$week, 3–4 days week^{-1}, almost everyday). [b]Further adjusted by quartile categories of salt intake. [c]Further stratified by PHC area. CI = confidence interval.

urinary excretion was only 0.12. The corresponding values in men were 0.49 and 0.38, respectively.

The associations with salt and salted food intake were always attenuated after stratifying by study area, where both salt/salted food intake and gastric cancer incidence varied significantly and were well correlated at the population level. Owing to the difficulties in estimating the intake of salt *per se*, its validity is expected to be lower within each study area with limited variation when compared with that in four areas combined. Therefore, stratification by study area may have underestimated the true association. Nonetheless, the associations were still clear with salt intake in men.

Among the salted foods, consumption of highly salted food such as salted fish roe and salted fish preserves were strongly associated with an increased risk of gastric cancer, even when adjusted for salt intake and stratified by study area both in men and women. The salt content of these salted foods is more than 5%; that of other salted foods such as miso soup, pickled vegetables and dried fish varied widely, but it is less than 5% in most foods. The observed findings might imply that either highly salted fish such as salted roe or salted preserves itself increased the risk of gastric cancer or that its intake was merely a good marker for a preference for salted foods or salt intake in general. Since 'almost daily' consumers of salted fish roe (2.2%) or salted fish preserves (0.9%) were limited, the intake frequency of these foods served to effectively categorise the subjects according to their preference for salted food intake. However, the intake frequencies of miso soup (77%) or pickled vegetables (49%) were relatively common, and their salt contents are different in each serving and each individual. Salt content of miso collected from 39 regions in 20 prefectures across Japan showed a wide variation, from 9.1 to 18.2% on the regional average (Watanabe *et al*, 1982) and those in miso soup preparation (18 g miso 200 g^{-1} soup in general recipe) varied individually. Salt contents of pickled vegetables also varied from less than 1% to over 10%. Even miso soup or pickled vegetables may have increased the risk of gastric cancer among subjects who generally consumed these types of foods with relatively higher salt content. An alternative explanation for the strong associations between highly salted foods and gastric cancer may be due to chemical caricinogens, which can be formed by reacting nitrate or nitrite in the process of preservation and of digestion in the stomach.

Table 5 Relative risks (RR) of incident gastric cancer by category of salted food intake at baseline in women: JPHC Study, 1990–2001

	Category of intake				
	Not daily	**1 cup day^{-1}**	**2 cups day^{-1}**	**3+ cups day^{-1}**	***P* for trend**
Miso soup					
Multivariate RR[a]	1	1.15	0.82	1.11	
95% CI	Reference	0.66–2.01	0.49–1.38	0.67–1.84	0.65
Multivariate RR[b]	1	1.15	0.79	0.85	
95% CI	Reference	0.64–2.09	0.40–1.55	0.39–1.87	0.63
Multivariate RR[c]	1	1.11	0.76	1.05	
95% CI	Reference	0.64–1.94	0.45–1.29	0.62–1.79	0.52
	Almost none	**1–2 days week^{-1}**	**3–4 days week^{-1}**	**Almost everyday**	
Pickled vegetables (e.g. shiozuke, nuka-misozuke)					
Multivariate RR[a]	1	1.01	2.2	1.74	
95% CI	Reference	0.44–2.31	1.05–4.58	0.89–3.41	0.05
Multivariate RR[b]	1	1.08	2.52	2.01	
95% CI	Reference	0.47–2.47	1.18–5.37	0.97–4.17	0.03
Multivariate RR[c]	1	0.92	1.77	1.32	
95% CI	Reference	0.39–2.13	0.78–3.99	0.60–2.91	0.46
Salted fish roe (e.g. tarako, suziko, ikura)					
Multivariate RR[a]	1	1.49	1.58	3.5	
95% CI	Reference	0.98–2.28	0.91–2.76	1.62–7.56	0.003
Multivariate RR[b]	1	1.59	1.63	3.37	
95% CI	Reference	1.02–2.47	0.90–2.96	1.48–7.66	0.006
Multivariate RR[c]	1	1.26	1.26	2.67	
95% CI	Reference	0.79–2.00	0.68–2.32	1.18–6.07	0.07
Salted fish preserves (e.g. shiokara, neri-uni)					
Multivariate RR[a]	1	0.95	2.6	2.56	
95% CI	Reference	0.62–1.45	1.44–4.69	0.63–10.5	0.03
Multivariate RR[b]	1	0.94	2.43	2.29	
95% CI	Reference	0.60–1.46	1.29–4.55	0.55–9.59	0.06
Multivariate RR[c]	1	0.83	2.18	2.18	
95% CI	Reference	0.54–1.27	1.19–3.98	0.53–8.98	0.14
Dried or salted fish (e.g. mezashi, shio-sake)					
Multivariate RR[a]	1	1.43	1.66	1.22	
95% CI	Reference	0.81–2.52	0.92–3.03	0.53–2.82	0.31
Multivariate RR[b]	1	1.48	1.68	1.17	
95% CI	Reference	0.82–2.65	0.89–3.16	0.49–2.80	0.44
Multivariate RR[c]	1	1.05	1.17	0.89	
95% CI	Reference	0.54–2.05	0.58–2.37	0.35–2.22	0.98

[a]Adjusted for age in 1990 (40–44, 45–49, 50–54, 55–59 years), cigarette smoking (never, past, current) and fruit and non-green–yellow vegetable intake (almost none, 1–2 days week^{-1}, 3–4 days week^{-1}, almost everyday). [b]Further adjusted by quartile categories of salt intake. [c]Further stratified by PHC area. CI = confidence interval.

Helicobacter pylori infection is closely associated with the risk of gastric cancer (Huang *et al*, 1998; Helicobacter and Cancer Collaborative Group 2001). The prevalence of *H. pylori* IgG antibody among randomly selected men aged 40–49 years was 76% in Ninohe, 86% in Yokote, 72% in Saku and 63% in Ishikawa PHC areas in our previous ecological study in 1989–1990 (Tsugane *et al*, 1994), which nearly paralleled age-adjusted incidence rates of gastric cancer in each area (Table 1). However, consumption of salted fish roe and salted fish preserves in these male cohort subjects was also closely associated with gastric cancer incidence at the population level: 5.8 and 4.3 days month^{-1} in Ninohe, 7.9 and 4.9 days month^{-1} in Yokote, 4.3 and 3.8 days month^{-1} in Saku and 0.9 and 1.0 days month^{-1} in Ishikawa (Tsugane *et al*, 2001). Moreover, a cross-sectional analysis in our ecological study showed that the intake of both pickled vegetable and miso soup was associated with *H. pylori* infection (Tsugane *et al*, 1994), although salted fish roe and salted fish preserves were not assessed in that study. An infection with *H. pylori*, however, is itself unlikely to increase the intake of salted foods. Therefore, even if *H. pylori* infection causes gastric cancer, restriction of salt and salted food intake can at least reduce its risk. It should be acknowledged that the worldwide decrease in the age-adjusted incidence of gastric cancer was obviously not due to the intentional eradication of *H. pylori* infection, but can be related to a reduction in salted food intake by use of refrigerators (World Cancer Research Fund in association with American Institute for Cancer Research, 1997). However, it is possible that improvement of sanitary condition and familial crowding may have been associated with unintentional falls in the prevalence of *H. pylori* infection and consequently gastric cancer incidence in recent birth cohorts especially in developed countries (Taylor and Blaser, 1991; International Agency for Research on Cancer, 1994).

To summarise, the intake of salt and salted food, especially highly salted food, was closely associated with the risk of gastric cancer. Considering that a substantial number of Japanese consume such foods daily and the relatively high risk involved, the restriction of these foods may be a practical way to prevent gastric cancer.

ACKNOWLEDGEMENTS

This study was supported by Grants-in-aid for cancer research and for the second term comprehensive 10-year strategy for cancer control from the Ministry of Health, Labor and Welfare of Japan. We thank all staff members in each study area and in the central office for their painstaking efforts to conduct the baseline survey and follow-up.

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Appendix

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British Journal of Nutrition (2004), **91**, 81–90
DOI: 10.1079/BJN20031024

Long-term vitamin C supplementation has no markedly favourable effect on serum lipids in middle-aged Japanese subjects

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(Received 24 February 2003 – Revised 8 September 2003 – Accepted 15 September 2003)

Antioxidant vitamins have been reported to be associated with an improvement in blood lipid profiles, but results are not consistent. The present study was designed to determine whether long-term vitamin C supplementation could alter serum lipid concentrations in subjects who completed a 5-year population-based double-blind intervention trial. A total of 439 Japanese subjects with atrophic gastritis initially participated in the trial using vitamin C and β-carotene to prevent gastric cancer. Before and upon early termination of β-carotene supplementation, 134 subjects dropped out of the trial; finally, 161 subjects assigned to the high-dose group (500 mg vitamin C/d) and 144 subjects assigned to the low-dose group (50 mg vitamin C/d) were studied. No favourable effect of vitamin C supplementation on serum concentrations of total cholesterol, HDL- and LDL-cholesterol, and triacylglycerol was observed, although high-dose vitamin C supplementation increased serum vitamin C concentrations substantially. Among women, the mean change in serum triacylglycerol decreased (−0·12 mmol/l, 95 % CI −0·32, 0·09) in the high-dose group, but increased (+0·12 mmol/l, 95 % CI 0·03, 0·22) in the low-dose group. In addition, the mean change in serum triacylglycerol among women with hypertriacylglycerolaemia was statistically significant (−1·21, 95 % CI −2·38, −0·05) after high-dose vitamin C supplementation. The 5-year vitamin C supplementation had no markedly favourable effects on the serum lipid and lipoprotein profile. However, our present results do not preclude the possibility that vitamin C supplementation may decrease triacylglycerol concentrations among women with hypertriacylglycerolaemia.

Vitamin C supplementation: Cholesterol: Triacylglycerol: Hypertriacylglycerolaemia: Intervention trial

The hypothesis that antioxidant vitamins may reduce the risk of CHD has been the focus of considerable research (Buring & Gaziano, 1997). Fundamental research (Lynch *et al.* 1996; Bok *et al.* 1999) and epidemiological studies (Trout, 1991; Carr & Frei, 1999) have provided evidence for mechanisms that may explain the effect of antioxidants on CHD. Two possible mechanisms of vitamin C in prevention of CHD have been postulated: (1) vitamin C may inhibit the oxidation of LDL-cholesterol both *in vivo* and *in vitro* (Steinberg & Chait, 1998); (2) improve the serum lipid levels by modulating the activity of several enzymes involved in lipid metabolism (e.g. cholesterol 7α-hydroxylase, 3-hydroxy-3-methylglutaryl-CoA reductase and lipoprotein lipase) (Bok *et al.* 1999).

Many epidemiological studies and a limited number of intervention trials (Jacques *et al.* 1995; Kurowska *et al.* 2000; Singhal *et al.* 2001) have provided evidence indicating that antioxidant vitamins are associated with an improvement in blood lipid and lipoprotein profile; however, the findings are not entirely consistent. Most authors using the non-pharmacological lipid-lowering approach (Jacques *et al.* 1995; Walden *et al.* 1997; Kurowska *et al.* 2000; Singhal *et al.* 2001) have studied small numbers of normolipidaemic subjects with short-term follow-up, mainly focused on dietary modification of fats and fatty acids, and their results have been inconsistent.

The Hiraka Chemoprevention Study (Tsubono *et al.* 1997; Kim *et al.* 2002) in Japan was originally designed as a population-based double-blind randomized controlled trial to examine the effect of vitamin C and/or β-carotene supplementation on the primary prevention of gastric cancer in a population at high-risk for gastric cancer and stroke. The objective of the present report was to examine the effect of 5-year vitamin C supplementation on serum lipid levels.

Abbreviation: TG, triacylglycerol.

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Methods

Subjects

The rationale, design and methodology of the study and the characteristics of the participants were published in detail by Tsubono *et al.* (1997) and Kim *et al.* (2002). Briefly, target subjects were men and women aged 40–69 years living in a village within the Yokote Public Health Centre district in Akita Prefecture, one of the regions with the highest mortality from stroke and gastric cancer in Japan (Tsugane *et al.* 1992). They regularly participated in annual screening programmes for circulatory diseases, conducted by each municipality under the National Health and Welfare Services Law for the Aged. Subject eligibility required: a diagnosis of chronic atrophic gastritis (defined as pepsinogen I <70 ng/ml and pepsinogen I:pepsinogen II ratio $<3{\cdot}0$); no past history of gastric cancer or related surgery; no previous history of liver cancer, cirrhosis, or of other cancers within the last 5 years; no abnormal liver function; no use of diet supplements containing β-carotene or vitamin C; no expectation of moving outside the study area within 1 year. Of 1231 individuals screened, 1214 provided serum for pepsinogen measurements, and 635 (52 %) were diagnosed with chronic atrophic gastritis. Thirty-three people were ineligible, since they failed to meet the inclusion criteria. Of the remaining 602 eligible individuals, 439 (73 %) consented to take part in the trial. Of the 439 persons initially accepted into the study, 134 subjects (seventy-three in the high-dose and sixty-one in the low-dose group) dropped out early, either before or upon the study protocol modification: the modification followed a report by Omenn *et al.* (1996) suggesting that β-carotene supplementation had potential harmful effects among individuals at high risk for lung cancer. Of the 305 remaining participants, 244 (124 in the high-dose and 120 in the low-dose group) completed the present study (Fig. 1).

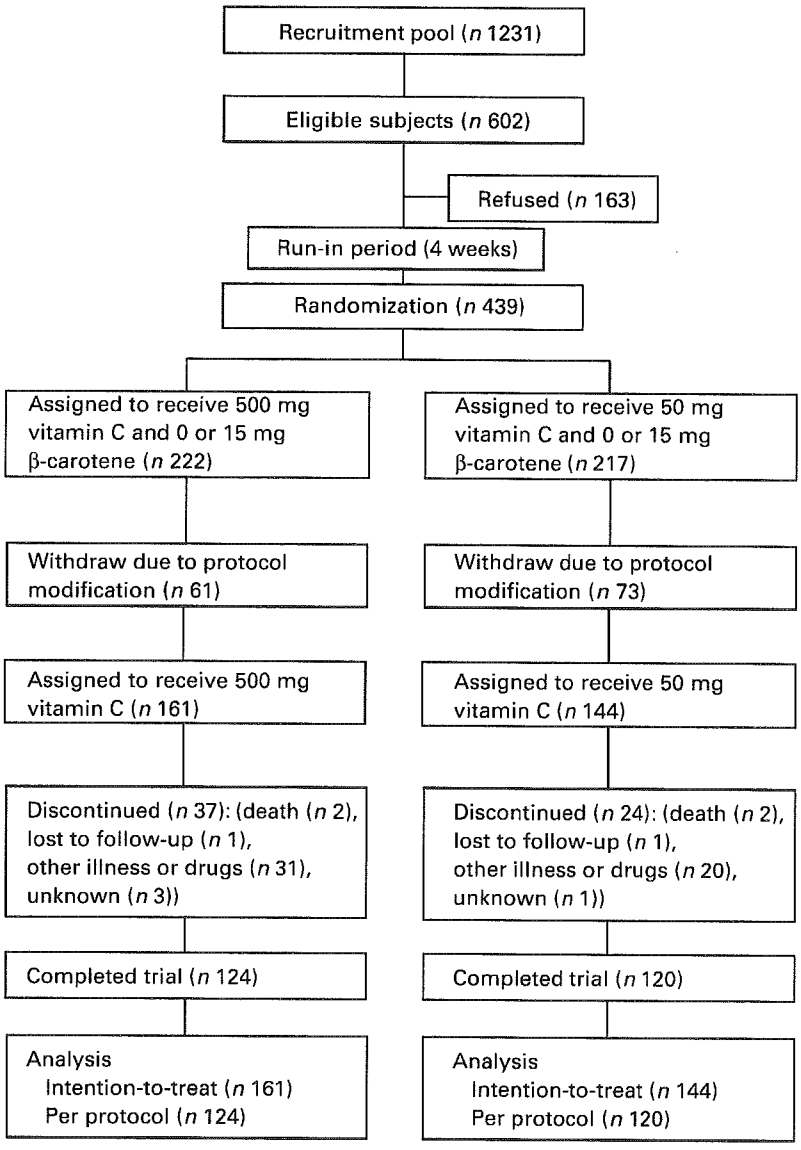

Fig. 1. Design of the randomized controlled diet.