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Consumption of sodium and salted foods in relation to cancer and cardiovascular disease: the Japan Public Health Center–based Prospective Study[1–4]

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ABSTRACT

Background: Although the influence of salt, per se, on the risk of cancer or cardiovascular disease (CVD) might differ from that of salt-preserved foods, few studies have simultaneously examined the effects of sodium and salted foods on the risk of either cancer or CVD.

Objective: We simultaneously examined associations between sodium and salted food consumption and the risk of cancer and CVD.

Design: During 1995–1998, a validated food-frequency questionnaire was administered to 77,500 men and women aged 45–74 y. During up to 598,763 person-years of follow-up until the end of 2004, 4476 cases of cancer and 2066 cases of CVD were identified.

Results: Higher consumption of sodium was associated with a higher risk of CVD but not with the risk of total cancer: multivariate hazard ratios for the highest compared with lowest quintiles of intake were 1.19 (95% CI: 1.01, 1.40; *P* for trend: 0.06) for CVD and 1.04 (95% CI: 0.93, 1.16; *P* for trend: 0.63) for total cancer. Higher consumption of salted fish roe was associated with higher risk of total cancer, and higher consumption of cooking and table salt was associated with higher risk of CVD. Similar results were seen for the risk of gastric or colorectal cancer and stroke.

Conclusions: Sodium intake as a whole salt equivalent may not increase the risk of cancer but may increase that of CVD. In contrast, salted food intake may increase the risk of cancer. Our findings support the notion that sodium and salted foods have differential influences on the development of cancer and CVD. *Am J Clin Nutr* 2010;91:456–64.

INTRODUCTION

Cancer and cardiovascular disease (CVD) are the leading causes of death in many parts of the world. Salt or processed foods with high salt concentrations or preservative content have been identified as risk factors for some cancers (1) and CVD (2). In previous observational studies, however, exposure from the consumption of salt as a whole might have been distinct from that of salt-preserved foods, which is explained as follows. Because the contribution of the salty seasonings used in cooking or at the table to sodium chloride intake was relatively small, the previous studies might have been unable to discern a difference between salt per se and salted food in their effect on these diseases. If so, any effect of salt on gastric cancer may have resulted primarily from the regular consumption of highly salt-concentrated preserved foods, rather than of total sodium chloride (1, 3). Furthermore, salt-preserved foods contain both potentially disadvantageous (eg, *N*-nitroso compounds) and advantageous factors [eg, n–3 (omega-3) polyunsaturated fatty acids, potassium, or antioxidants (1, 2, 4, 5)]. Despite these possible differences in the effect of salt-preserved foods and of total sodium from salty seasonings and salt-preserved foods on the risk of total cancer and CVD, most studies to date have evaluated sodium or salted food consumption in relation to the risk of site-specific cancer or CVD separately.

One approach to determine the effect of salt consumption as a whole sodium chloride equivalent or as salted food consumption on disease risk is to examine their associations with the risk of cancer and CVD simultaneously in the same population. To our knowledge, however, no such prospective cohort study has been reported. Because of the difficulty of estimating habitual salt or sodium intake, few studies have simultaneously examined the effects of sodium and salted foods on the risk of either cancers [2 studies for gastric cancer (6, 7)] or CVD [7 studies, for sodium only (8–14)]. Asian populations tend to differ from Western populations with respect to the distribution of exposure (higher

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consumption of sodium and salt-preserved fish) and outcomes (higher incidence of gastric cancer and stroke). Moreover, the contribution of salty seasonings used in cooking or at the table to total salt intake (excluding miso) is relatively high, at 52% in Japan (15). Studies that aim to characterize the influence of sodium and salted food consumption on the risk of cancer and CVD in Asian populations are therefore important.

In this study, we used a validated, comprehensive food-frequency questionnaire (FFQ) with estimation of habitual cooking and table salt to examine associations between sodium and salted foods and the risk of cancer and CVD in a population-based prospective cohort study in Japan. Particular focus was placed on the intake of sodium and specific salt-preserved foods.

SUBJECTS AND METHODS

Study population

The Japan Public Health Center–based Prospective Study was conducted in 2 cohorts, one initiated in 1990 (cohort I) and the other in 1993 (cohort II). The study design has been described in detail previously (16). The study protocol was approved by the Institutional Review Board of the National Cancer Center, Tokyo, Japan.

The study population was defined as all registered Japanese inhabitants in 11 public health center areas, aged 40–59 y in cohort I and aged 40–69 y in cohort II, who were identified by the population registries maintained by the local municipalities. Two public health center areas (Tokyo and Osaka) were excluded from the present analysis because either cancer or CVD incidence data, or both, were not available.

Surveys of the cohort participants by self-administered questionnaire were conducted twice, the first in 1990 (cohort I) and 1993 (cohort II), and the second in 1995 (cohort I) and 1998 (cohort II). Because the second survey included more comprehensive information on food intake frequency than the first, the second survey was used as the starting point to assess dietary exposure in the present study. The questionnaire also included information on medical history and lifestyle factors, such as smoking and alcohol drinking.

After the exclusion of 9272 persons who had died, moved out of a study area, or were lost to follow-up before the starting point, the 107,400 subjects who remained were eligible for participation. Of these, 91,225 subjects responded (42,761 men and 48,464 women; response rate 84.9%) and were included in the present study.

Food-frequency questionnaire

The FFQ asked about the usual consumption of 138 foods and beverages, which included 4 seasonings (dressing, mayonnaise, Worcestershire sauce, and ketchup), during the previous year in standard portions/units and 9 frequency categories (17). Standard portion sizes were specified for each food item in 3 amount choices: small (50% smaller than standard), medium (same as standard), and large (50% larger than standard). The amount of each food consumed (g/d) was calculated from the responses. Energy and nutrient intake, including sodium, were calculated with the use of the *Standardized Tables of Food Composition, fifth revised edition* (18). Sodium intake from cooking salt and soy sauce was estimated for 3 food groups (meats, fish, and vegetables) from the responses for dietary and cooking behaviors, with cooking salt for 6 cooking methods (raw, stewed, grilled, deep-fried, stir-fried, and other) that have specified salt content (0.8–1.5%) multiplied by the individual intake of each food group according to the cooking methods most frequently used by the individual. In addition, table salt and soy sauce added to these food groups were taken into account for sodium intake according to specified salt content (0–0.5%) for the 3 frequency categories (19). Miso soup consumption was calculated with the use of 6 frequency categories and 9 categories for the number of bowls per day, which ranged from <1 to ≥ 10 bowls/d, and was further adjusted for taste preference by multiplying by the specified coefficient for mild, common, and strong taste preferences of 0.75, 1.0, and 1.3, respectively.

The following food items were considered as salted foods in our analysis: pickled vegetables (6 items: Chinese radishes, green leafy vegetables, plums, Chinese cabbage, cucumbers, and eggplant; 1.5–7.6% salt content); dried and salted fish [3 items: salted fish (salted codfish or atka mackerel or salmon); *himono* (dried and salted Japanese horse mackerel); *shirasuboshi* (dried young sardines); 1.7–4.1%]; salted fish roe [one item included 2 descriptions: *tarako* (salted Alaska pollack roe) or *suziko* (salted salmon roe); 4.6–4.8%]; and miso soup (1%). Salt content data for specific food items other than miso soup were taken from the *Standardized Tables of Food Composition, fifth revised edition* (18). The weighting ratios for miso soup composition, which consists of miso and cooking water, were 8% and 92%, respectively, and were obtained from the dietary records (19).

The validity of the FFQ for the assessment of sodium intake has been confirmed (20). Spearman's correlation coefficients between energy-adjusted sodium intake based on the FFQ and those based on 28-d (or 14-d for the Ishikawa public health center area) dietary records among subsamples ($n = 215$ and 350 for cohorts I and II, respectively) of men and women were 0.47 and 0.50 for cohort I and 0.32 and 0.31 for cohort II, respectively. Correlation coefficients for the reproducibility of the FFQ administered 1 y apart for men and women were 0.49 and 0.63 for cohort I and 0.56 and 0.67 for cohort II, respectively (21, 22). To examine the accuracy of habitual sodium intake based on the FFQ over an extended period, correlation coefficients between energy-adjusted sodium consumption based on the FFQ and creatinine-adjusted sodium concentrations based on 2 measurements of 24-h urinary excretion at a 7-mo interval for men and women were 0.42 and 0.30, respectively, among subsamples of cohort I (20).

Follow-up

Subjects were followed from the starting point until 31 December 2004. Changes in residence status, including survival, were obtained annually from the residential registry in each area or, for those who had moved out of the study area, through the municipal office in the area to which they had moved. Mortality data for persons in the residential registry are forwarded to the Ministry of Health, Labor and Welfare and are coded for inclusion in the national Vital Statistics. Residency registration and death registration are required by the Basic Residential Register Law and Family Registry Law, respectively, and the registries are thought to be complete. During the follow-up period in the

present study, 5419 (5.9%) subjects died, 2634 (2.9%) moved out of the study area, and 19 (0.02%) were lost to follow-up.

The occurrence of cancer was identified by active patient notification from major local hospitals in the study area and from data linkage with population-based cancer registries, with permission from the local governments responsible for the cancer registries. Cancer cases were coded in accordance with the *International Classification of Diseases for Oncology, third edition* (23). In our cancer registry system, the proportion of cases for which information was available from death certificates only was 5.0%. Diagnoses of myocardial infarction according to the criteria of the MONICA (Monitoring Trends and Determinants of Cardiovascular Disease) project (24) and diagnoses of stroke according to the criteria of the National Survey of Stroke (25) were confirmed for all cases by either or both computer tomographic scan and magnetic resonance imaging as recorded in the medical record and reviewed by hospital or public health center physicians in each registered major local hospital in each public health center area (26, 27). CVD cases with a death certificate or self-report only, without confirmation by medical records, were treated as non-CVD cases. CVD was defined as myocardial infarction or stroke, whichever occurred first. We confirmed 7056 cases of newly diagnosed cancers and 3349 cases of CVD among the 91,225 subject by 31 December 2004.

Statistical analysis

Of the 91,225 respondents, we excluded subjects with a history of cancer or coronary heart disease or stroke (n = 8,165) and those who did not complete the diet component of the questionnaire (n = 1482). Subjects with a history of these conditions were defined as diagnosed with cancer or CVD before the starting point or from self-reports in the questionnaire. Of the 81,578 subjects, 4078 who reported extreme total energy intake (lower and upper 2.5 percentiles: 910 and 4000 kcal/d, respectively) were excluded, which left 77,500 subjects (35,730 men, 41,770 women) for final analysis, including 4476 with cancer (867 gastric cancer, 836 colorectal cancer, 541 lung cancer, 304 breast cancer, 271 liver cancer, 1657 other cancers) and 2066 with CVD (1745 stroke, 338 myocardial infarction). Participants with both cancer and CVD were included in both analyses. We performed separate analyses for major site-specific cancers (gastric, colorectal, or lung cancer: 50% of total cancer cases) and stroke or myocardial infarction. Of the CVD cases, participants with both stroke and myocardial infarction were included in both analyses.

Person-years of follow-up were calculated for each subject from the starting point to the date of diagnosis, date of emigration from the study area, date of death, or end of the follow-up period (31 December 2004), whichever occurred first. Subjects lost to follow-up were censored at the last confirmed date of presence in the study area. A total of 593,620 person-years were accrued for the cancer analysis and 598,763 for the CVD analysis.

Hazard ratios (HRs) and 95% CIs were calculated for the categories of energy-adjusted sodium and salted food consumption in quintiles for men and women combined, with the lowest consumption category as the reference, with the use of Cox proportional hazards models with adjustment for potential confounding variables according to the SAS PHREG procedure (SAS software, version 9.1; SAS Institute Inc, Cary, NC). A residual model was used for energy adjustment of sodium and salted food consumption (28).

We conducted the initial analyses by adjusting for sex and age at the starting point (5-y groups). In the multivariate model, we further adjusted for body mass index (BMI; in kg/m^2) (<19, 19–22.9, 23–24.9, 25–26.9, and ≥27), smoking status (never, past, and current), alcohol consumption (none, occasional, or 1–149, 150–299, 300–449, and ≥450 g ethanol/wk), physical activity in metabolic equivalent task-hours/d (<30, 30–34.9, 35–39.9, and ≥40), and quintiles of total energy, potassium (as a proxy for the intake of fruit and vegetables for the analysis of cancers) and calcium intake (29, 30). We did not adjust for area in the models, because salt or salted food consumption was substantially defined by area, and adjustment may therefore have masked the true influence of salt or salted food on gastric cancer or stroke, which accounted for the largest part of total cancer (19.5%) and CVD (84.5%), respectively (31–34). Subjects for whom values for any of the potential confounders were missing were excluded from the multivariate analysis (7079 were excluded, which left 70,421 in the analyses), because findings did not materially differ when subjects with missing values were retained in the analyses by assigning dummy variables for missing responses. We also assessed linear associations with the use of the median values of sodium or salted food intake for each quintile.

Because the distribution of sodium consumption differed by sex, we also performed a stratified analysis according to sex-specific quintile of sodium or salted food consumption. We additionally performed subgroup analyses according to smoking status ("never" as nonsmoker or "past" and "current smoker" as ever smoker), age (<60 or ≥60 y), cohort (I or II), BMI (<25 or ≥25), and alcohol intake (<300 or ≥300 g ethanol/wk). All P values were 2-sided, and statistical significance was determined at $P < 0.05$.

RESULTS

Contributions to gross sodium intake in this population from pickled vegetables, dried and salted fish, salted fish roe, miso soup, and cooking and table salt were 11.1%, 3.4%, 0.7%, 18.8%, and 38.8%, respectively, and the correlation coefficients between sodium and these foods were 0.46, 0.24, 0.18, 0.43, and 0.71, respectively. Sodium intake ranged from a median value of 3084 mg/d in the lowest quintile to 6844 mg/d in the highest. Subjects with higher sodium consumption were slightly older.

Age-adjusted values for subject characteristics according to quintile of sodium consumption are shown in **Table 1**. Subjects with higher consumption were less likely to be men, drinkers, and ever smokers, and more likely to consume higher amounts of potassium and calcium. Higher sodium intake was not associated with levels of physical activity or prevalence of ever smoking or overweight.

Whereas no association was shown between sodium or cooking and table salt consumption and cancer, higher consumption of salted fish roe was significantly associated with a higher risk of total cancer, as shown in **Table 2**. Furthermore, the HR of total cancer was significantly higher for the highest quintile of dried and salted fish than the lowest, albeit without a linear trend. A significant positive association was shown between sodium consumption and risk of CVD, as well as

TABLE 1
Characteristics of subjects according to quintile of sodium intake: Japan Public Health Center–based Prospective Study, 1995 and 1998 (n = 77,500)[1]

	Quintile of sodium intake					
	Lowest	Second	Third	Fourth	Highest	P for trend[2]
Median intake						
Sodium (mg)	3084	4005	4709	5503	6844	—
Salt equivalent (g)	7.8	10.2	11.9	13.9	17.3	—
Subjects (n)	15,500	15,500	15,500	15,500	15,500	—
Men	9570	8050	7129	6032	4949	—
Women	5930	7450	8371	9468	10551	—
Age (y)[3]	56.1 ± 8.0	56.4 ± 7.8	56.7 ± 7.7	57.1 ± 7.6	57.9 ± 7.6	—
Salted food intake (g/d)[4]						
Pickled vegetables	16.6 (0.33)	24.9 (0.33)	32.5 (0.33)	42.1 (0.33)	70.8 (0.33)	<0.01
Dried and salted fish	12.2 (0.19)	16.1 (0.19)	19.0 (0.19)	22.0 (0.19)	27.5 (0.19)	<0.01
Salted fish roe	0.8 (0.03)	1.3 (0.03)	1.6 (0.03)	2.0 (0.03)	2.5 (0.03)	<0.01
Miso soup	133 (1.23)	201 (1.23)	243 (1.23)	282 (1.23)	331 (1.23)	<0.01
Cooking and table salt	2.8 (0.07)	3.8 (0.07)	4.5 (0.07)	5.5 (0.07)	7.9 (0.07)	<0.01
BMI ≥25 kg/m^2 (%)[5]	28.2	28.0	28.7	29.8	31.1	<0.01
Past smoker (%)[5]	10.5	9.1	8.5	7.3	6.1	<0.01
Current smoker (%)[5]	31.4	27.4	25.1	21.4	19.4	<0.01
Moderate drinker, >0 to <300 g ethanol/wk (%)[5]	22.4	24.0	24.0	22.0	19.8	<0.01
Heavy drinker, ≥300 g ethanol/wk (%)[5]	27.6	18.2	13.5	9.6	6.0	<0.01
Physical activity (MET-h/d)[4]	33.56 (0.05)	33.69 (0.05)	33.79 (0.05)	33.85 (0.05)	33.77 (0.05)	<0.01
Dietary intake[4]						
Energy (kcal/d)	1959 (5.08)	2009 (5.08)	2019 (5.07)	1999 (5.08)	1958 (5.06)	0.41
Potassium (mg/d)	2322 (5.45)	2647 (5.45)	2805 (5.44)	2978 (5.45)	3338 (5.43)	<0.01
Sodium:potassium ratio	1.37 (0.01)	1.60 (0.01)	1.77 (0.01)	1.94 (0.01)	2.26 (0.01)	<0.01
Calcium (mg/d)	499 (1.91)	534 (1.91)	546 (1.91)	565 (1.91)	606 (1.91)	<0.01

[1] MET-h, metabolic equivalent task hours.
[2] Trend tests across categories of sodium consumption were calculated by ANCOVA for age-adjusted means and the Cochran-Mantel-Haenszel test for age-adjusted proportions.
[3] Values are means ± SDs.
[4] Values are age-adjusted means; SEs in parentheses.
[5] Values are age-standardized proportions.

between cooking and table salt and CVD, whereas no positive association was shown between any specific salted food item and risk of CVD. On the contrary, an inverse association was shown between dried and salted fish and CVD risk.

On additional analysis that used major site-specific cancers (gastric and colorectal cancer) and stroke or myocardial infarction as endpoints (**Table 3**), higher consumption of pickled vegetables was associated with a higher risk of gastric cancer, whereas higher consumption of dried and salted fish and salted fish roe was associated with a higher risk of both gastric cancer and colorectal cancer (although the linear trend was not significant for gastric cancer risk according to dried and salted fish intake). In contrast, no association was shown between sodium or cooking and table salt consumption and any major site-specific cancer, including gastric cancer. A significant positive association was shown between sodium consumption as well as cooking and table salt and risk of stroke, whereas no positive association was shown between any salted food and risk of stroke. Higher consumption of sodium or cooking salt was not significantly associated with a higher risk of myocardial infarction, whereas an inverse association was shown between dried and salted fish intake and risk of myocardial infarction. These associations with salted foods other than cooking and table salt were not changed after adjustment for sodium intake, whereas the positive association between cooking and table salt intake and risk of stroke was attenuated to the null (data not shown).

Results for stratified analyses according to sex-specific quintiles of sodium or salted food consumption were similar to those obtained with sex-combined quintiles. Specifically, no association was shown between sodium consumption and risk of total cancer for either sex, with HRs that corresponded for the highest compared with lowest quintiles of intake of 1.11 (95% CI: 0.96, 1.28; P for trend: 0.23) and 0.94 (95% CI: 0.79, 1.11; P for trend: 0.45) for men and women, respectively. Higher consumption of salted fish roe among women and pickled vegetables among men and women was nonsignificantly associated with a higher risk of total cancer (data not shown). CVD risk was positively but nonsignificantly associated with sodium consumption for both men (HR: 1.18, 95% CI: 0.96, 1.45; P for trend: 0.15) and women (HR: 1.16, 95% CI: 0.91, 1.47; P for trend: 0.08) as well as with cooking and table salt consumption (data not shown). The only salted food positively associated with CVD risk among women was salted fish roe. On the contrary, higher consumption of dried and salted fish was associated with lower risk of CVD among men (data not shown). Tests of interaction were not statistically significant between sex and sodium, or any salted food intake for the risk of cancers or CVDs (data not shown). The results did not materially differ in analyses stratified by smoking status, age, cohort, BMI, or alcohol intake (data not shown).

We also conducted analyses that excluded subjects who reported medication use for hypertension, diabetes, or hyperlipidemia.

TABLE 2
Hazard ratios (HRs) and 95% CIs for total cancer and cardiovascular disease according to quintiles (Q) of sodium and salted food consumption: Japan Public Health Center–based Prospective Study, 1995 and 1998–2004 (n = 77,500)

		Total cancer				Cardiovascular disease			
	Median intake[1]	Person- years	No. of cases	HR[2] (95% CI)	HR[3] (95% CI)	Person- years	No. of cases	HR[2] (95% CI)	HR[3] (95% CI)
	g								
Sodium									
Q1	3084	113,841	876	1.00 (reference)	1.00 (reference)	114,800	416	1.00 (reference)	1.00 (reference)
Q2	4005	117,226	881	1.01 (0.92, 1.11)	1.02 (0.93, 1.13)	118,218	428	1.03 (0.90, 1.17)	1.11 (0.96, 1.29)
Q3	4709	118,923	906	1.05 (0.95, 1.15)	1.07 (0.96, 1.18)	119,983	386	0.93 (0.81, 1.07)	1.02 (0.87, 1.19)
Q4	5503	121,521	882	1.02 (0.93, 1.12)	1.01 (0.91, 1.12)	122,609	403	0.97 (0.84, 1.11)	1.10 (0.94, 1.29)
Q5	6844	122,109	931	1.08 (0.99, 1.19)	1.04 (0.93, 1.16)	123,153	433	1.04 (0.90, 1.19)	1.19 (1.01, 1.40)
P for trend				0.10	0.61			0.78	0.06
Pickled vegetables									
Q1	3.3	115,909	833	1.00 (reference)	1.00 (reference)	116,584	458	1.00 (reference)	1.00 (reference)
Q2	12	116,861	844	1.04 (0.95, 1.15)	1.04 (0.94, 1.15)	117,789	373	0.83 (0.73, 0.96)	0.88 (0.76, 1.01)
Q3	23	118,303	844	1.03 (0.93, 1.13)	1.01 (0.91, 1.12)	119,163	424	0.93 (0.81, 1.06)	0.98 (0.85, 1.13)
Q4	39	120,344	978	1.15 (1.05, 1.26)	1.15 (1.04, 1.27)	121,649	397	0.83 (0.72, 0.95)	0.88 (0.76, 1.02)
Q5	85	122,203	977	1.13 (1.03, 1.25)	1.08 (0.97, 1.20)	123,579	414	0.85 (0.74, 0.97)	0.92 (0.79, 1.07)
P for trend				<0.01	0.10			0.07	0.48
Dried and salted fish									
Q1	0.5	115,798	901	1.00 (reference)	1.00 (reference)	116,548	499	1.00 (reference)	1.00 (reference)
Q2	6.4	121,052	924	1.10 (1.01, 1.21)	1.08 (0.98, 1.19)	122,066	433	0.93 (0.82, 1.06)	0.97 (0.85, 1.12)
Q3	13	119,264	896	1.09 (0.99, 1.20)	1.05 (0.95, 1.16)	120,540	366	0.80 (0.70, 0.91)	0.84 (0.72, 0.97)
Q4	23	118,975	820	1.01 (0.92, 1.11)	0.99 (0.89, 1.10)	119,998	380	0.84 (0.73, 0.95)	0.88 (0.76, 1.02)
Q5	43	118,531	935	1.13 (1.03, 1.24)	1.11 (1.00, 1.22)	119,610	388	0.83 (0.73, 0.95)	0.86 (0.74, 0.99)
P for trend				0.10	0.19			0.01	0.04
Salted fish roe									
Q1	0.0	113,158	880	1.00 (reference)	1.00 (reference)	114,116	420	1.00 (reference)	1.00 (reference)
Q2	0.2	113,450	875	1.07 (0.97, 1.17)	1.08 (0.96, 1.22)	114,249	424	1.08 (0.95, 1.24)	1.02 (0.86, 1.21)
Q3	0.7	118,606	877	1.05 (0.96, 1.16)	1.05 (0.94, 1.17)	119,674	421	1.05 (0.92, 1.20)	1.00 (0.86, 1.17)
Q4	1.6	121,082	874	1.14 (1.04, 1.26)	1.12 (1.01, 1.25)	122,147	378	1.03 (0.90, 1.19)	1.00 (0.85, 1.17)
Q5	4.7	127,323	970	1.18 (1.07, 1.29)	1.15 (1.04, 1.27)	128,577	423	1.06 (0.93, 1.22)	1.08 (0.93, 1.25)
P for trend				<0.01	0.01			0.71	0.27
Miso soup									
Q1	42	113,632	765	1.00 (reference)	1.00 (reference)	114,404	380	1.00 (reference)	1.00 (reference)
Q2	132	114,185	876	1.09 (0.99, 1.21)	1.08 (0.97, 1.20)	115,262	374	0.93 (0.81, 1.07)	0.99 (0.85, 1.16)
Q3	218	119,013	934	1.10 (1.00, 1.21)	1.09 (0.98, 1.20)	120,142	403	0.94 (0.82, 1.08)	0.97 (0.83, 1.13)
Q4	313	122,733	932	1.05 (0.95, 1.15)	1.03 (0.93, 1.14)	124,078	390	0.87 (0.75, 1.00)	0.90 (0.77, 1.04)
Q5	458	124,057	969	1.01 (0.92, 1.11)	0.99 (0.89, 1.10)	124,876	519	1.07 (0.94, 1.22)	1.09 (0.95, 1.26)
P for trend				0.60	0.36			0.30	0.35
Cooking and table salt									
Q1	2.3	117,214	973	1.00 (reference)	1.00 (reference)	118,305	437	1.00 (reference)	1.00 (reference)
Q2	3.4	117,997	884	0.95 (0.87, 1.04)	0.97 (0.88, 1.07)	118,906	430	1.03 (0.90, 1.18)	1.05 (0.91, 1.21)
Q3	4.4	118,323	896	0.99 (0.91, 1.09)	1.02 (0.92, 1.12)	119,392	409	1.01 (0.88, 1.15)	1.06 (0.92, 1.23)
Q4	5.6	119,446	845	0.94 (0.86, 1.03)	0.96 (0.86, 1.06)	120,547	370	0.91 (0.79, 1.05)	0.98 (0.84, 1.15)
Q5	8.0	120,640	878	1.02 (0.92, 1.12)	1.00 (0.89, 1.12)	121,613	420	1.08 (0.94, 1.23)	1.21 (1.03, 1.42)
P for trend				0.68	0.94			0.60	0.05

[1] Values for sodium intake are provided in milligrams.
[2] HRs were adjusted for sex and age (5-y groups). Linear trends across quintiles of sodium or salted food intake were tested with the use of the median consumption for each quintile as an ordinal variable.
[3] HRs were further adjusted for BMI (in kg/m^2 ; <19, 19–22.9, 23–24.9, 25–26.9, ≥27), smoking status (never, past, current), alcohol consumption (none; occasional; 1–149, 150–299, 300–449, ≥450 g ethanol/wk), physical activity in metabolic equivalent task-hours/d (<30, 30–34.9, 35–39.9, ≥40), and quintiles of energy, potassium, and calcium.

Although the results for total cancer and major site-specific cancers were not substantially changed (data not shown), multivariate HRs of CVD and stroke for the highest compared with lowest quintiles of sodium intake were greater than those before the exclusion of these patients (HR for CVD: 1.30, 95% CI: 1.06, 1.60; *P* for trend: 0.01; HR for stroke: 1.36, 95% CI: 1.09, 1.71; *P* for trend < 0.01).

DISCUSSION

In this population-based prospective cohort study in Japan, we observed that higher consumption of sodium as a whole was associated with an increased risk of CVD but not of cancer. In contrast, higher consumption of salted fish roe was associated with a higher risk of cancer but not of CVD. Moreover, higher consumption of dried and salted fish was associated with a lower

TABLE 3
Hazard ratios (HRs) and 95% CIs for major site-specific cancers and stroke or myocardial infarction according to quintiles (Q) of sodium and salted food consumption: Japan Public Health Center–based Prospective Study, 1995 and 1998–2004 (n = 77,500)

	Gastric cancer		Colorectal cancer		Stroke		Myocardial infarction	
	No. of cases	HR[1] (95% CI)	No. of cases	HR[1] (95% CI)	No. of cases	HR[1] (95% CI)	No. of cases	HR[1] (95% CI)
Sodium								
Q1	177	1.00 (reference)	164	1.00 (reference)	355	1.00 (reference)	64	1.00 (reference)
Q2	175	1.05 (0.84, 1.31)	161	1.05 (0.84, 1.33)	349	1.05 (0.90, 1.24)	83	1.50 (1.05, 2.14)
Q3	167	1.06 (0.84, 1.34)	163	1.08 (0.85, 1.37)	315	0.97 (0.82, 1.14)	73	1.34 (0.92, 1.96)
Q4	174	1.05 (0.83, 1.34)	171	1.08 (0.84, 1.37)	343	1.08 (0.92, 1.28)	64	1.26 (0.85, 1.88)
Q5	174	1.07 (0.83, 1.38)	177	1.10 (0.85, 1.42)	383	1.21 (1.01, 1.43)	54	1.09 (0.71, 1.68)
P for trend		0.64		0.51		0.03		0.91
Pickled vegetables								
Q1	95	1.00 (reference)	163	1.00 (reference)	375	1.00 (reference)	86	1.00 (reference)
Q2	175	1.91 (1.47, 2.48)	136	0.91 (0.72, 1.15)	315	0.92 (0.78, 1.07)	62	0.74 (0.52, 1.04)
Q3	168	1.70 (1.30, 2.22)	190	1.20 (0.96, 1.50)	354	1.00 (0.86, 1.17)	75	0.94 (0.67, 1.31)
Q4	212	2.14 (1.65, 2.77)	182	1.11 (0.88, 1.40)	343	0.92 (0.79, 1.08)	55	0.69 (0.48, 1.00)
Q5	217	2.24 (1.71, 2.93)	165	0.95 (0.74, 1.22)	358	0.96 (0.81, 1.13)	60	0.75 (0.51, 1.10)
P for trend		<0.01		0.71		0.80		0.22
Dried and salted fish								
Q1	129	1.00 (reference)	158	1.00 (reference)	416	1.00 (reference)	86	1.00 (reference)
Q2	195	1.56 (1.22, 1.98)	156	1.12 (0.88, 1.42)	362	0.96 (0.83, 1.12)	74	1.06 (0.76, 1.48)
Q3	199	1.57 (1.24, 2.00)	171	1.18 (0.93, 1.49)	301	0.83 (0.71, 0.97)	69	0.93 (0.65, 1.31)
Q4	173	1.48 (1.15, 1.89)	157	1.15 (0.90, 1.46)	319	0.87 (0.74, 1.02)	63	0.97 (0.68, 1.38)
Q5	171	1.46 (1.14, 1.88)	194	1.40 (1.11, 1.77)	347	0.90 (0.77, 1.06)	46	0.66 (0.44, 0.98)
P for trend		0.12		<0.01		0.22		0.03
Salted fish roe								
Q1	146	1.00 (reference)	158	1.00 (reference)	343	1.00 (reference)	80	1.00 (reference)
Q2	144	1.14 (0.85, 1.51)	135	0.90 (0.68, 1.20)	354	1.05 (0.87, 1.27)	78	0.95 (0.63, 1.43)
Q3	181	1.35 (1.06, 1.72)	162	1.01 (0.79, 1.29)	345	1.02 (0.86, 1.21)	76	0.85 (0.58, 1.23)
Q4	170	1.39 (1.09, 1.77)	178	1.22 (0.96, 1.56)	333	1.08 (0.91, 1.28)	47	0.63 (0.42, 0.95)
Q5	226	1.66 (1.32, 2.09)	203	1.35 (1.07, 1.69)	370	1.14 (0.97, 1.34)	57	0.80 (0.55, 1.16)
P for trend		<0.01		<0.01		0.09		0.21
Miso soup								
Q1	145	1.00 (reference)	140	1.00 (reference)	317	1.00 (reference)	67	1.00 (reference)
Q2	158	1.04 (0.82, 1.33)	159	1.12 (0.88, 1.42)	302	0.95 (0.81, 1.13)	73	1.17 (0.82, 1.67)
Q3	170	1.09 (0.86, 1.39)	180	1.11 (0.88, 1.41)	350	1.00 (0.85, 1.18)	55	0.80 (0.54, 1.17)
Q4	181	1.06 (0.84, 1.35)	177	1.05 (0.82, 1.33)	335	0.91 (0.78, 1.08)	59	0.83 (0.57, 1.21)
Q5	213	1.10 (0.87, 1.39)	180	1.01 (0.80, 1.28)	441	1.12 (0.96, 1.31)	84	0.98 (0.69, 1.39)
P for trend		0.45		0.73		0.14		0.47
Cooking and table salt								
Q1	210	1.00 (reference)	180	1.00 (reference)	368	1.00 (reference)	75	1.00 (reference)
Q2	173	0.96 (0.78, 1.19)	178	1.09 (0.88, 1.36)	349	1.02 (0.87, 1.20)	84	1.17 (0.84, 1.64)
Q3	175	1.01 (0.81, 1.26)	155	0.98 (0.77, 1.23)	345	1.06 (0.90, 1.24)	66	1.02 (0.71, 1.47)
Q4	143	0.86 (0.68, 1.09)	156	0.97 (0.76, 1.23)	316	0.98 (0.83, 1.16)	56	0.97 (0.66, 1.43)
Q5	166	1.03 (0.80, 1.33)	167	1.01 (0.78, 1.31)	367	1.21 (1.02, 1.44)	57	1.12 (0.74, 1.69)
P for trend		1.00		0.78		0.05		0.88

[1] HRs were adjusted for sex, age (5-y groups), BMI (in kg/m^2; <19, 19–22.9, 23–24.9, 25–26.9, ≥27), smoking status (never, past, current), alcohol consumption (none; occasional; 1–149, 150–299, 300–449, ≥450 g ethanol/wk), physical activity in metabolic equivalent task-hours/d (<30, 30–34.9, 35–39.9, ≥40), and quintiles of energy, potassium, and calcium. Linear trends across quintiles of sodium or salted food intake were tested with the use of the median consumption for each quintile as an ordinal variable.

risk of CVD. To our knowledge, this is the first prospective cohort study to simultaneously examine associations between sodium and salted foods and the risk of cancer and CVD.

Results from 7 previous prospective cohort studies that examined the association between sodium intake and CVD risk have been poorly consistent: 3 studies showed significant associations between sodium and risk (8, 9, 11), 1 showed a significant inverse association (12), and 3 showed no association (10, 13, 14). These studies used different methods to assess exposure: 2 used 24-h urinary sodium excretion (9, 10), 1 used a validated FFQ that consisted of 35 items (8), and 4 used a single 24-h dietary recall method (11–14). Our results are consistent with 2 of the 3 studies that used an FFQ or 24-h urinary excretion to assess habitual salt intake (8, 9).

Only one previous study has examined the association between salted foods and total cancer risk (35), although at a small scale (155 cases). Results showed no association between consumption of salted fish, Japanese pickles, and miso soup with total

cancer mortality. With regard to gastric cancer, the 6 prospective studies of the association of this cancer with total salt intake after adjustment for other risk factors (6, 7, 36–39) were inconsistent, although the report of a joint World Cancer Research Fund/American Institute for Cancer Research Expert Consultation identified salt as a "probable" risk of gastric cancer (1): 2 of 3 studies conducted in Japan showed a positive association between salt intake and gastric cancer incidence (6, 38) and 3 conducted in Western countries [Norway (7), Netherlands (37), and the United States (39)] showed no association, whereas the third study conducted in Japan reported an inverse association between total salt intake and gastric cancer mortality. Of these studies, one conducted in the Japan Public Health Center–based cohort reported positive associations with the risk of gastric cancer for both salt intake and salted foods but did not take table salt or cooking method into consideration when total salt intake was calculated (6). In contrast, the present study did include table salt or cooking method variables, which correlated strongly with total sodium intake ($r = 0.71$), and showed them to represent 40% of total sodium intake. Nevertheless, we observed no association between total sodium consumption and the risk of either total cancer or gastric cancer.

The major strength of the present study was its prospective design, which avoided exposure recall bias. Other strengths included the following: study subjects were defined as the general population; response rate to the questionnaire (85%) was acceptable for study settings such as this; and the proportion of losses to follow-up (0.02%) was negligible. Furthermore, the use of a general population in Asia and an FFQ enabled sodium intake to be estimated from not only salted foods but also salty seasonings from dietary and cooking behaviors, which likely represented a relatively large portion of sodium intake. In the present study, this strength may have eliminated the possibility that the observed absence of an association between sodium intake as a whole salt equivalent and gastric cancer incidence was attributable to any inability to take into account salty seasonings from dietary and cooking behaviors in the estimation of total sodium intake.

The present results suggest that the associations of cancer with specific foods with high salt concentrations, such as salted fish roe, are not due to the amount of salt per se, but rather to other causes. Consistent with our results, Tsugane et al (6) reported that, after adjustment for total salt intake, higher consumption of salted fish roe among men and women and of dried or salted fish among men was associated with a higher risk of gastric cancer. One potential explanation for this may have been the presence of chemical carcinogens such as *N*-nitroso compounds in dried fish or salted fish roe, which can be formed by the reaction of nitrate or nitrite in the curing process or in the body (1, 4, 5), or heterocyclic amines, which have been detected in fish or meat cooked in high temperatures, such as grilling (40), which is commonly used for dried and salted fish in Japan. An additional, inseparable explanation is the destruction of the gastric mucosal barrier by a high intragastric salt concentration, which leads to inflammation, diffuse erosion, and degeneration. The subsequent proliferative change may exacerbate the effect of food-derived carcinogens (3, 41). In contrast, the decreased risk of CVD with dried and salted fish intake might reflect beneficial cardiovascular effects of n–3 polyunsaturated fatty acids in fish in the inhibition of platelet aggregation, modulation of the inflammatory system, and lowering of blood pressure (27). Salted foods might thus not be as precise a surrogate marker of total salt in the investigation of the influence on cancer and CVD as whole sodium chloride consumption.

Our study has several potential limitations. First, with the use of multiple 24-h urinary sodium excretion as a reference, the validity of the FFQ for sodium intake was moderate at best [$r = 0.30$–0.42 (20)]. Some misclassification in the FFQ was unavoidable, and it is possible that the accuracy of salt intake was less than that of salted food intake (6). If this biased the association between sodium and cancers toward the null, then the observed association would have underestimated the true magnitude of the association between sodium and CVD as well as cancers. Second, variation in sodium consumption among subjects was also moderate at best, with median intake in the highest quintile group (6844 mg) only 2.2-fold that in the lowest (3084 mg; Table 1). However, this range was similar to that of a study based on 24-h urinary excretion, which identified positive associations between sodium intake and CVD [1.6-fold difference (9)]. Given that this variation was sufficient to detect an association between sodium intake and CVD risk, the possibility that the lack of association between sodium intake and total and gastric cancer was due to insufficient variation therefore appears unlikely. In addition, we did not obtain information on infection with *Helicobacter pylori*, a strong risk factor for gastric cancer (42). Because salted food intake may increase the risk of *H. pylori* infection, the prevalence of infection would have been higher in subjects with higher intakes of salt or salted food (43). Therefore, even if *H. pylori* infection causes gastric cancer, we believe it unlikely that the failure to account for *H. pylori* infection masked a positive association between the consumption of sodium and the risk of gastric cancer.

In conclusion, this population-based prospective cohort study in Japan showed that the amount of sodium as a whole salt equivalent was not associated with the risk of cancer but was associated with an increased risk of CVD. In contrast, the intake of highly salt-concentrated preserved foods may increase the risk of cancer. Our findings support the notion that sodium and salted foods have differential influences on the development of cancer and CVD.

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Plasma levels of C-reactive protein and serum amyloid A and gastric cancer in a nested case–control study: Japan Public Health Center-based prospective study

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Gastric carcinogenesis may be under the combined influence of factors related to the host, *Helicobacter pylori* bacterial virulence and the environment. One possible host-related factor is the inflammatory or immune response. To clarify this point, we investigated the association between plasma levels of C-reactive protein (CRP) and serum amyloid A (SAA) and the subsequent risk of gastric cancer in a population-based nested case–control study. Subjects were observed from 1990 to 2004. Among 36 745 subjects who answered the baseline questionnaire and provided blood samples, 494 gastric cancer cases were identified and matched to 494 controls for our analysis. The overall distribution of CRP and SAA was not apparently associated with the development of gastric cancer. However, a statistically significant increased risk was observed when subjects were categorized dichotomously. The adjusted odds ratio (OR) for the development of gastric cancer for the CRP-positive group (CRP > 0.18 mg/dl) compared with the CRP-negative group was 1.90 [95% confidence interval (CI): 1.19–3.02, $P = 0.007$]. The OR for the SAA-positive group (SAA > 8 μg/ml) compared with the SAA-negative group was 1.93 (95% CI: 1.22–3.07, $P = 0.005$). In conclusion, our results suggest that those who react strongly to inflammation or who have a high host immune response, as reflected by extremely elevated plasma levels of CRP and SAA, are at a high risk to develop gastric cancer.

Introduction

It is well established that cancer arises in chronically inflamed tissue, and one of the classic examples is *Helicobacter pylori*-associated gastric cancer (1). *Helicobacter pylori* persistently colonizes the gastric mucosa, leading to chronic inflammation, atrophic gastritis and, finally, gastric cancer. There are high interindividual differences in the extent of gastric inflammation among *H.pylori*-infected subjects, and only a small proportion of them develop clinical consequences. This indicates that gastric carcinogenesis may be under the combined influence of factors related to the host, bacterial virulence and the environment. One possible host-related factor is the inflammatory or immune response. Many studies have reported an association between serum proinflammatory cytokines [e.g. interleukin (IL)-6, IL-8 and IL-1β] levels (2–4) or polymorphisms (such as IL-1, IL-2 and IL-8) and gastric cancer risk (5–8), but the results are controversial. The lack of consensus may be partly due to the nature of cytokines, which are components of a large, complex signaling network, and difficulties in measuring their levels and interactions. Measurement of cytokines in plasma is difficult because of their short plasma half-lives and the presence of blocking factors (9). Additionally, combinations of cytokines have been found to have additive, inhibitory or synergistic effects. Therefore, more useful or systematic indicators of host inflammatory or immune response are needed.

C-reactive protein (CRP) is a well-established indicator of inflammation in the body (10). It is an acute-phase reactant that reflects low-grade systemic inflammation and has been studied in a variety of cardiovascular diseases. CRP production by the liver is regulated by cytokines, principally IL-6 and tumor necrosis factor α, which is the main trigger for the production of IL-6 by a variety of cells. In fact, strong positive associations between IL-6, tumor necrosis factor α and CRP were observed (11). Serum amyloid A (SAA) is another major acute-phase reactant. It is a putative serum precursor of the amyloid A protein, which constitutes amyloid fibrils in secondary amyloidosis and is an apolipoprotein associated with the high density lipoprotein 3 fraction of serum (12). In most studies, a parallel increase of SAA and CRP has been observed, although some studies have delineated acute-phase SAA as the more sensitive parameter (13,14). Therefore, to indicate the host inflammatory or immune response systematically, CRP and SAA may be useful markers.

In this large-scale nested case–control study, we aimed to examine whether the host inflammatory or immune response has any association with the development of gastric cancer. To clarify this point, we explored the relation of plasma levels of CRP and SAA to risk of developing gastric cancer. As far as we know, this is the first study to prospectively seek this association in a population.

Materials and methods

Study population

The Japan Public Health Center-based prospective study (JPHC Study) is an ongoing cohort study to investigate cancer, cardiovascular disease and other lifestyle-related diseases. The first group (Cohort I) of the JPHC Study was started in 1990 and the second group (Cohort II) in 1993 (15). The JPHC Study included 140 420 subjects (68 722 men and 71 698 women), defined as all inhabitants in the study areas [27 cities, towns or villages served by 11 public health centers (PHCs)] who were 40–59 years old (Cohort I) or 40–69 years old (Cohort II). Among the study subjects, those registered at one PHC area in Cohort I were excluded from the present analysis because data on cancer incidence were not available. Additionally, one subcohort in Cohort II was excluded because the selection of subjects differed from that of other cohort subjects, i.e. random sampling of residents from a municipality population registry for one city, stratified by 10 year age–gender groups. We thus defined 123 576 subjects (61 009 men and 62 567 women) for the present study. The JPHC Study was approved by the institutional review board of the National Cancer Center, Tokyo, Japan.

Baseline survey

In 1990 for Cohort I and in 1993–1994 for Cohort II, subjects were asked to reply to a lifestyle questionnaire that covered sociodemographic characteristics, medical history, smoking and drinking habits, diet and so on. Details of the food frequency questionnaire included in the baseline survey have been described previously (16). A total of 99 808 (81%) subjects—47 525 men and 52 283 women—responded to the questionnaires.

We excluded subjects who self-reported cancer at baseline ($n = 2136$), those who were not Japanese ($n = 18$) and those who were later discovered to have moved away at baseline ($n = 11$). This left 97 644 eligible subjects (46 803 men and 50 841 women). Among them, 36 745 subjects (38%; 13 467 men and 23 278 women) donated blood samples at health checkups conducted by the PHC in each area. Each subject voluntarily provided 10 ml of blood during the health checkups. As customary, subjects were asked to avoid having a meal later than 21:00 on the day before the examination. The last time of either consuming a meal or drinking water or tea was recorded. The plasma and buffy layer were divided into four tubes, with each tube holding 1.0 ml (3 tubes for plasma and 1 for the buffy layer) and stored at 80°C. Blood was collected from 1990 to 1992 in Cohort I and from 1993 to 1995 in Cohort II.

Follow-up and identification of gastric cancer

In Japan, at the time the study was conducted, a PHC played a role as an organization that provided primary health care, including health checkups,

Abbreviations: BMI, body mass index; CagA, cytotoxin-associated gene A; CI, confidence interval; CRP, C-reactive protein; ICD-O, International Classification of Diseases for Oncology; Ig, immunoglobulin; IL, interleukin; JPHC, Japan Public Health Center; OR, odds ratio; PG, pepsinogen; PHC, public health center; SAA, serum amyloid A.

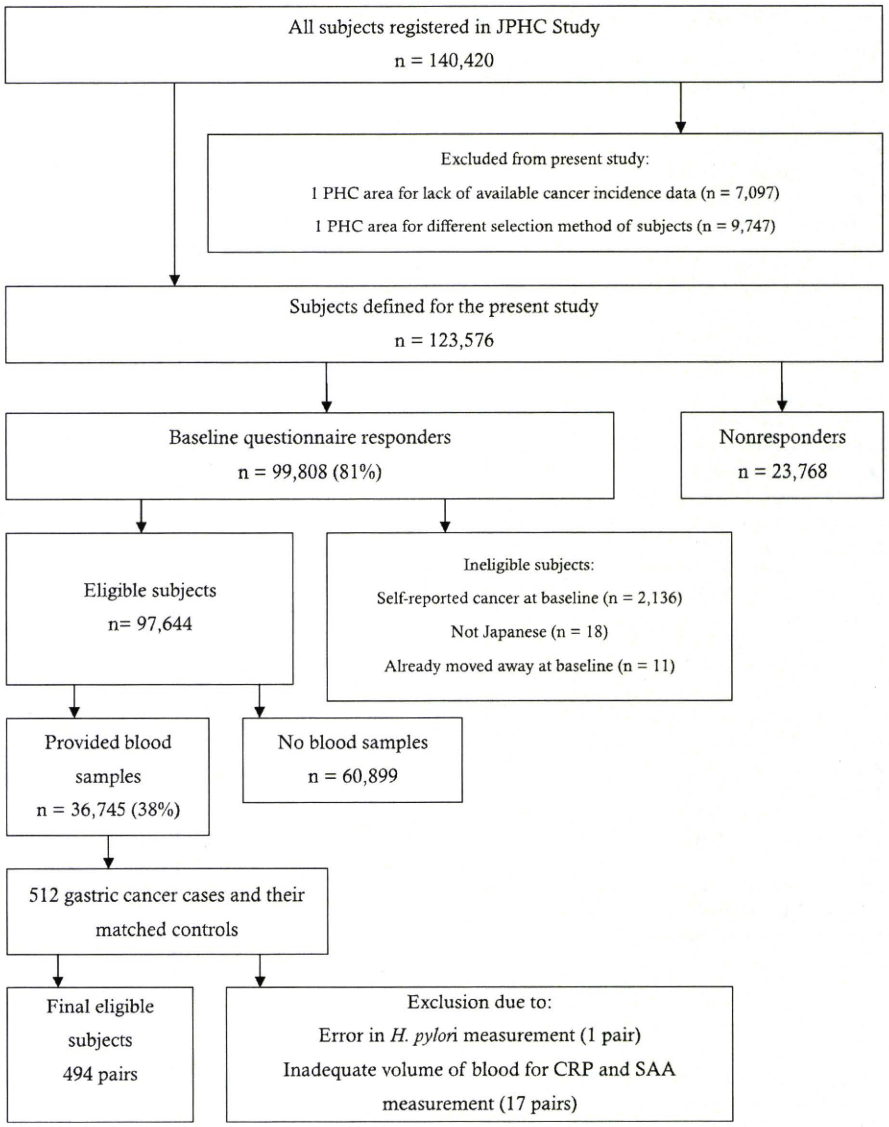

Fig. 1. Flow of study population.

or other health promotion activities for all inhabitants of the municipalities supervised by the PHC. In this study, the main role of the PHC was to collect and report data on mortality, relocation and cancer cases.

Death and relocation

We observed study subjects until 31 December 2004. The changes in residency status, including death, were identified annually through the residential registry in each area. To confirm causes of death, we used mortality data from the Ministry of Health, Labour and Welfare. Residence and death registration are required by law in Japan, and the registries are believed to be complete. Among 36 745 study subjects, 1423 (3.9%) moved away from the study area, 1610 (4.4%) died and 11 (0.03%) were lost to follow-up within the study period.

Cancer registry for JPHC Study

Data on newly diagnosed cases of cancer were collected from two sources: active patient notification from the local major hospitals in the study area and data linkage with population-based registries (usually prefecture-wide). Death certificate information was used as a supplementary information source. In our cancer registry system, the proportion of cases of gastric cancer for which information was based on death certificate notification was 7.6% and on information available from death certificates only was 2.1%. This level of quality for the information was considered satisfactory for the present study.

Identification of gastric cancer and selection of control subjects

Cases of gastric cancer were extracted from the cancer registry for the JPHC Study on the basis of site [International Classification of Diseases for Oncology (ICD-O) code C160–169] (17). Up to the end of the study period, 512 new gastric cancer cases were identified. Until quite recently in Japan, the upper third of the stomach has been called the 'cardia' on the basis of the guidelines for gastric cancer classification (18). Because it seemed difficult to distinguish the cardia, which is mainly located in the esophagogastric junction, from the upper third of the stomach, we combined tumors at these sites into one group for analysis (ICD-O code C160–161). A tumor located on the lower side of the stomach was classified as distal gastric cancer (ICD-O code C162–167). Subsites that could not be classified because of a diffuse lesion (ICD-O code C168) or those with no information (ICD-O code C169) were categorized as an unclassified subsite. Histologic classification was based on one author's (S.S.) review, in consultation with a pathologist, of the record reported by each hospital. The subdivisions were made on the basis of a classification derived by Lauren (19). For each case, one control was selected from subjects who had no history of gastric cancer and who lived in the study area when the case was

diagnosed. Each control was matched to a case for gender, age (±3 years), PHC area, blood donation date (±2 months) and fasting time at blood donation (±5 h). Because of a technical error in measurement of *H.pylori* and inadequate volume of blood available for CRP and SAA measurements, 1 case with its matched control and another 17 pairs (8 cases with their matched controls and 10 controls with their matched cases) were excluded. Finally, we had 494 sets each of cases and controls for use in the present analysis. A flowchart of the study subjects is provided in Figure 1.

Laboratory analysis

CRP and SAA concentrations were determined by the latex agglutination nephelometric immunoassay test (LZ test 'Eiken' CRP-HG; Eiken Kagaku Co. Ltd, Tokyo, Japan; and LZ test 'Eiken' SAA; Eiken Kagaku Co. Ltd, respectively). For the CRP test, based on 10 replicated measurements of three concentrations of blood samples (0.07, 0.50 and 4.41 mg/dl) at the time of analyses, the coefficients of variation were 1.69%, 0.59% and 0.76%, respectively. For SAA, 10 replicated measurements of two concentrations of blood samples (22 μg/ml and 110 μg/ml) yielded a coefficient of variation of reproducibility values of 1.53% and 1.17%. Normal values for the examined parameters were <0.18 mg/dl for CRP and <8 μg/ml for SAA according to the kit's protocol. Both cutoff values were based on data from reports for the same kit. The cutoff value of CRP was set by the iterative truncation method among 478 health checkup samples (20). In brief, after repeated deletion of outliers, mean ± 1.96 SD was considered the normal range. For SAA, after being converted to a logarithm, the value was set as the upper 95th percentile of the distribution of 1056 normal subjects (0–70 years old) (21).

Immunoglobulin (Ig) G antibodies to *H.pylori* were measured with a direct enzyme-linked immunosorbent assay kit (E Plate 'Eiken' *H.pylori* Antibody; Eiken Kagaku Co. Ltd). Levels of IgG were categorized as seropositive and seronegative for *H.pylori* according to a selective cutoff value (≤10 or >10). The cutoff value was based on the results of sensitivity and specificity calculated with the urea test, which is the gold standard (report by company). Assays of cytotoxin-associated gene A (CagA) were performed with the use of an enzyme-linked immunosorbent assay kit, in which horseradish peroxidase was used as the enzyme tracer (CagA IgG EIA; Sceti Co. Ltd, Rome, Italy). According to the manufacturer's protocol, samples with IgG values ≤10 RU/ml must be considered non-reactive for anti-CagA IgG antibodies; samples with IgG values within 10–15 RU/ml must be considered weakly reactive and samples with IgG values >15 RU/ml must be considered reactive for anti-CagA IgG antibodies. With regard to interpretation of these results, reactive and/or questionable samples are considered positive for anti-CagA IgG antibodies, i.e. values >10 are regarded as CagA positive. Serum levels of pepsinogen I and II (PGI and PGII, respectively) were measured by commercial kits based on a two-step enzyme immunoassay (E Plate 'Eiken' Pepsinogen I; Eiken Kagaku Co. Ltd; and E Plate 'Eiken' Pepsinogen II; Eiken Kagaku Co. Ltd). Results were defined as 'atrophic' when the criteria of both PGI level ≤70 ng/ml and PGI : PGII ratio ≤3.0 were fulfilled. Comparing the PG levels between gastric cancer cases and healthy controls retrospectively, Miki (22) reported that applying a PGI level ≤70 ng/ml and a PGI : PGII ratio ≤3.0 as cutoff values was most effective in distinguishing cases from controls. Using these criteria, other authors have showed an extremely high correlation (r = 0.999) between atrophy and age-adjusted gastric cancer mortality among inhabitants of five areas in Japan (23). Among atrophic cases, more severe cases with a PGI level ≤30 ng/ml and PGI : PGII ratio ≤2.0 were defined as severe atrophy.

All measurements were conducted by a person blinded to the case–control situation.

Statistical analysis

Statistical analysis included chi-square test, analysis of variance, analysis of covariance and conditional logistic model. Multiple conditional logistic regression analyses were conducted to control for potential confounding factors. For cardia cancer, smoking status, alcohol consumption (for SAA analysis), intake of salt, body mass index (BMI), family history of gastric cancer, history of infectious or inflammatory disease (i.e. cardiovascular disease, ischemic heart disease, liver disease and kidney disease) and current use of analgesics for lumbago, neuralgia, common cold, arthrosis and joint pain were controlled. For all gastric cancer, all non-cardia cancer, differentiated-type non-cardia cancer and undifferentiated-type non-cardia cancer further adjustment was applied for *H.pylori* infection, atrophy and CagA seropositivity. Smoking status was divided into four groups: never smoker, past smoker, current smoker with <20 cigarettes per day and current smoker with ≥20 cigarettes per day). Alcohol consumption was defined as drinker (>1 day/week) and non-drinker (<1 day/week). BMI was categorized into three groups so that each category included an approximately equal number of controls. Salt was treated as a continuous variable. Family history of gastric cancer was regarded as positive if at least one parent or sibling had gastric cancer. CRP and SAA status (positive/negative) were determined according to the protocol's normal value. Additionally, the non-linear continuous models of the association between CRP and SAA and gastric cancer risk were tested by PROC GAM. Odds ratios (ORs) were calculated relative to the cutoff points of CRP and SAA. Because the distribution was skewed, log transformation was conducted for CRP, SAA, *H.pylori* titer, CagA titer, PGI level and PGII and PGI : PGII ratio, which altered the distribution close to normal in comparisons of the mean values between groups.

Reported P-values were two sided, and all statistical analyses were done with SAS software version 9.1 (SAS Institute Inc., Cary, NC).

Results

Baseline characteristics of cases and controls are shown in Table I. Among listed factors, predominance of *H.pylori* positivity, CagA status, atrophy and family history of gastric cancer were apparent in cases compared with controls.

Table II summarizes the distribution of lifestyle factors and plasma biomarkers according to the CRP and SAA status among controls. Forty-seven (9.5%) and 63 (12.8%) subjects met the criteria for being positive for plasma CRP and SAA, respectively. For CRP status, no factors were differently distributed other than SAA levels; the mean value of SAA among CRP-positive subjects was >10 times that of CRP-negative subjects ($P < 0.0001$). Plasma CRP level among SAA-positive subjects was 13 times that among SAA-negative subjects ($P < 0.0001$). Correlation of the log-transformed CRP and SAA was 0.55 ($P < 0.0001$). Mean daily salt intake was higher in SAA-negative subjects compared with SAA-positive subjects. This may be due to the predominance of male gender and alcohol consumption among SAA-negative subjects, which contribute to high salt intake. When gender and alcohol consumption were adjusted (analysis of covariance), the difference in salt intake was no longer significant ($P = 0.40$). Compared with positive subjects, SAA-negative subjects had a significantly higher *H.pylori* titer against IgG antibody and more frequent distribution of male gender, alcohol consumption, *H.pylori* positivity and atrophy.

Table I. Baseline characteristics of cases and controls

	Case	Control	P-value[a]
n	494	494	
Age	57.3 (0.3)	57.3 (0.3)	Matching value
Men (%)	329 (66.6%)	329 (66.6%)	Matching value
Cigarette smoking			
Never smoker (%)	228 (46.2%)	245 (49.6%)	
Past smoker (%)	91 (18.4%)	98 (19.8%)	
Current smoker with <20 cigarettes per day (%)	133 (26.9%)	109 (22.1%)	
Current smoker with ≥20 cigarettes per day (%)	42 (8.5%)	42 (8.5%)	0.35
Alcohol consumption			
Never or occasional (%)	245 (49.6%)	244 (49.4%)	
≥1 day, <300 g/week (%)	187 (37.9%)	203 (41.1%)	
≥1 day, ≥300 g/week (%)	62 (12.6%)	47 (9.5%)	0.26
BMI			
<25	396 (80.2%)	369 (74.7%)	
25–29.9	89 (18.0%)	113 (22.9%)	
≥30	9 (1.8%)	12 (2.4%)	0.12
Family history of gastric cancer (%)	60 (12.2%)	40 (8.1%)	0.03
Salt (g/day)	5.3 (0.1)	5.1 (0.1)	0.40
Helicobacter pylori positive (%)[b]	463 (93.7%)	371 (75.1%)	<0.0001
Helicobacter pylori positive (%)[c]	489 (99.0%)	445 (90.1%)	<0.0001
CagA (+) (%)	375 (75.9%)	346 (70.0%)	0.04
Atrophy (%)	406 (82.2%)	285 (57.7%)	<0.0001

Values are mean (SE) except where specified otherwise.
[a]Based on chi-square test or analysis of variance.
[b]Based on IgG antibody.
[c]Based on CagA positive and/or *Helicobacter pylori* IgG antibody positive.

Table II. Distribution of lifestyle factors and plasma biomarkers according to CRP and SAA status among control

	CRP status			SAA status		
	Negative (CRP ≤ 0.18 mg/dl)	Positive (CRP > 0.18 mg/dl)	*P*-value[a]	Negative (SAA ≤ 8 μg/ml)	Positive (SAA > 8 μg/ml)	*P*-value[a]
n	447	47		431	63	
Age	57.1 (0.3)	58.6 (1.1)	0.20	57.2 (0.3)	58.1 (0.9)	0.35
Men (%)	296 (66.2%)	33 (70.2%)	0.58	296 (68.7%)	33 (52.4%)	0.01
BMI						
<25	336 (75.2%)	33 (70.2%)		327 (75.9%)	42 (66.7%)	
25–29.9	100 (22.4%)	13 (27.7%)		93 (21.6%)	20 (31.8%)	
≥30	11 (2.5%)	1 (2.1%)	0.71	11 (2.6%)	1 (1.6%)	0.19
Cigarette smoking						
Never smoker (%)	225 (50.3%)	20 (42.6%)		207 (48.0%)	38 (60.3%)	
Past smoker (%)	91 (20.4%)	7 (14.9%)		90 (20.9%)	8 (12.7%)	
Current smoker with <20 cigarettes per day (%)	97 (21.7%)	12 (25.5%)		98 (22.7%)	11 (17.5%)	
Current smoker with ≥20 cigarettes per day (%)	34 (7.6%)	8 (17.0%)	0.12	36 (8.4%)	6 (9.5%)	0.23
Alcohol consumption						
Never or occasional (%)	218 (48.8%)	26 (55.3%)		204 (47.3%)	40 (63.5%)	
≥1 day, <300 g/week (%)	184 (41.2%)	19 (40.4%)		185 (42.9%)	18 (28.6%)	
≥1 day, ≥300 g/week (%)	45 (10.1%)	2 (4.3%)	0.39	42 (9.7%)	5 (7.9%)	0.05
Family history of gastric cancer (%)	36 (8.1%)	4 (8.5%)	0.91	36 (8.4%)	4 (6.4%)	0.85
Salt (g/day)	5.2 (0.1)	4.9 (0.3)	0.43	5.2 (0.1)	4.6 (0.3)	0.04
CRP (mg/dl)/SAA (μg/ml)[b]	3.6 (1.8)	38.6 (5.6)	<0.0001[a]	0.05 (0.03)	0.65 (0.07)	<0.0001[a]
Helicobacter pylori positive (%)[c]	338 (75.6%)	33 (70.2%)	0.42	332 (77.0%)	39 (61.9%)	0.01
Helicobacter pylori positive (%)[d]	403 (90.2%)	42 (89.4%)	0.86	390 (90.5%)	55 (87.3%)	0.43
Helicobacter pylori titer	43.9 (2.3)	36.1 (7.1)	0.31[e]	44.0 (2.3)	37.1 (6.1)	0.02[e]
CagA (+) (%)	314 (70.3%)	32 (68.1%)	0.76	302 (70.1%)	44 (69.8%)	0.97
CagA titer	85.1 (4.2)	74.7 (12.9)	0.72[e]	84.6 (4.3)	80.8 (11.1)	0.82[e]
PGI	28.6 (0.8)	29.7 (2.5)	0.55[e]	28.5 (0.8)	30.1 (2.1)	0.52[e]
PGII	11.2 (0.3)	10.8 (1.0)	0.60[e]	11.2 (0.3)	11.0 (0.8)	0.71[e]
PGI : PGII	3.5 (0.6)	2.9 (1.8)	0.83[e]	3.5 (0.6)	3.2 (1.6)	0.28[e]
Atrophy (%)	260 (58.2%)	25 (53.2%)	0.51	256 (59.4%)	29 (46.0%)	0.04
Severe atrophy (%)	122 (27.3%)	9 (19.2%)	0.23	119 (27.6%)	12 (19.1%)	0.15

Values are mean (SE) except where specified otherwise.
[a]Based on chi-square test or analysis of variance.
[b]Mean plasma CRP level for SAA status and mean plasma SAA level for CRP status.
[c]Based on IgG antibody.
[d]Based on CagA positive and/or *Helicobacter pylori* IgG antibody positive.
[e]Based on analysis of variance of log biomarkers.

In Table III, ORs and 95% confidence intervals (CIs) of CRP positivity for development of gastric cancer are presented by tumor subsite and histologic types. CRP ranged from 0 to 19.1 mg/dl (mean: 0.14 mg/dl, median: 0.033 mg/dl) among cases and from 0 to 9.3 mg/dl (mean: 0.13 mg/dl, median: 0.032 mg/dl) among controls. The risk of developing gastric cancer increased by ~36% among those who were CRP positive; the crude OR equaled 1.36 (95% CI: 0.91–2.02, $P = 0.13$), although with no significance. After being adjusted for potential confounding variables, the point estimate altered substantially and reached the level of statistical significance; the adjusted OR equals 1.90 (95% CI: 1.19–3.02, $P = 0.007$). Among the adjusted covariates, *H.pylori* infection contributed the most to the elevation of risk; adding only *H.pylori* infection to the model elevated the OR to 1.67, which was much higher than the OR for adding CagA seropositivity (adjusted OR = 1.39), atrophy (adjusted OR = 1.48) or even all other lifestyle factors [i.e. cigarette smoking, BMI, family history, history of infectious or inflammatory disease, current drug use of analgesics and salt intake (adjusted OR = 1.47)]. When the cancers were stratified by tumor location and histologic type, the largest OR was demonstrated for cardia cancers, but it failed to reach statistical significance; adjusted OR equaled 3.14 (95% CI: 0.51–19.39, $P = 0.22$). Among non-cardia cancers, the association did not differ much by histologic type. When the analyses were repeated with subjects divided into quartiles according to control distribution of the CRP level (<0.012, 0.012–0.032, 0.032–0.081 and ≥0.081 mg/dl), no apparent association was observed. Compared with the lowest (reference) group, the adjusted ORs (95% CIs) for development of gastric cancer for the second, the third and the highest group were 0.85 (0.56–1.29), 0.96 (0.62–1.47) and 1.35 (0.88–2.07), respectively (P for trend = 0.0496). When CRP was treated as a continuous measure, the adjusted OR for development of gastric cancer was 1.06 (0.87–1.28), for 1 mg/dl increase of log-transformed CRP. Furthermore, non-linear continuous models did not reveal any evidence of dose response.

SAA among cases and controls ranged from 0 to 319.7 μg/ml (mean: 5.9 μg/ml, median: 2.6 μg/ml) and from 0 to 847.5 μg/ml (mean: 7.0 μg/ml, median: 2.5 μg/ml), respectively. For SAA positivity, about a 2-fold increased risk was observed for total gastric cancer and non-cardia cancer; the adjusted ORs (95% CIs) were 1.93 (1.22–3.07, $P = 0.005$) and 2.13 (1.14–3.98, $P = 0.02$), respectively (Table IV). Among adjusted covariates, atrophy as well as *H.pylori* infection contributed most of the elevation of risk. Among non-cardia cancers, no difference was observed by histologic type. The largest OR was demonstrated for cardia cancers, although it failed to reach the level of statistical significance; the adjusted OR equaled 3.84 (95% CI: 0.82–17.99, $P = 0.09$). When results for SAA status were shown separately for men and women, there was no material difference; the adjusted ORs for developing total gastric cancer were 1.95 and 2.15 for men and women, respectively. The adjusted OR for cardia cancer among women could not be calculated because of the small sample size; therefore, all analyses were conducted for men and women combined. No apparent association was observed when SAA

Table III. ORs and 95% CIs of CRP positivity (CRP > 0.18 mg/dl) for development of gastric cancer by tumor subsite and histologic type

	No. of CRP-positive cases/controls	Crude OR (95% CI)	*P*-value	Adjusted OR (95% CI)[a]	*P*-value
All (494 pairs)	62/47	1.36 (0.91–2.02)	0.13	1.90 (1.19–3.02)	0.007
Cardia (39 pairs)	7/2	3.50 (0.73–16.85)	0.12	3.14 (0.51–19.39)	0.22
Non-cardia (355 pairs)	44/33	1.36 (0.85–2.16)	0.20	2.18 (1.24–3.84)	0.007
Differentiated type (232 pairs)	30/23	1.32 (0.76–2.29)	0.33	1.77 (0.89–3.52)	0.10
Undifferentiated type (107 pairs)	9/8	1.14 (0.41–3.15)	0.80	2.01 (0.53–7.62)	0.30

[a]Cardia cancers, adjusted for cigarette smoking, BMI, family history of gastric cancer, history of infectious or inflammatory disease, current drug use of analgesics and salt intake. All gastric cancers, all non-cardia cancers, differentiated-type non-cardia cancer and undifferentiated-type non-cardia cancer, further adjusted for *Helicobacter pylori* infection, CagA positivity and atrophy.

Table IV. ORs and 95% CIs of SAA positivity (SAA > 8 μg/ml) for development of gastric cancer by tumor subsite and histologic type

	No. of SAA-positive cases/controls	Crude OR (95% CI)	*P*-value	Adjusted OR (95% CI)[a]	*P*-value
All (494 pairs)	75/63	1.26 (0.86–1.86)	0.24	1.93 (1.22–3.07)	0.005
Cardia (39 pairs)	11/5	3.00 (0.81–11.08)	0.10	3.84 (0.82–17.99)	0.09
Non-cardia (355 pairs)	45/39	1.21 (0.74–2.00)	0.45	2.13 (1.14–3.98)	0.02
Differentiated type (232 pairs)	27/25	1.11 (0.59–2.06)	0.75	1.73 (0.81–3.72)	0.16
Undifferentiated type (107 pairs)	12/11	1.14 (0.41–3.15)	0.80	1.80 (0.41–7.92)	0.44

[a]Cardia cancers, adjusted for cigarette smoking, alcohol consumption, BMI, family history of gastric cancer, history of infectious or inflammatory disease, current drug use of analgesics and salt intake. All gastric cancers, all non-cardia cancers, differentiated-type non-cardia cancer and undifferentiated-type non-cardia cancer, further adjusted for *Helicobacter pylori* infection, CagA positivity and atrophy.

level was divided into quartiles (<1.3, 1.3–2.5, 2.5–5.1 and ≥5.1 μg/ml). Compared with the lowest (reference) group, the adjusted ORs (95% CIs) for development of gastric cancer for the second, the third and the highest group were 0.81 (0.53–1.24), 1.06 (0.70–1.61) and 1.19 (0.77–1.85), respectively ($P = 0.20$). When SAA was treated as a continuous measure, the adjusted OR for development of gastric cancer was 1.00 (0.995–1.00) for 1 mg/dl increase of log-transformed SAA. Similar to the analysis of CRP, non-linear continuous models did not reveal any evidence of dose response.

Because of the high correlation between CRP and SAA, we included only the values for the marker being analyzed (Tables III and IV). When CRP and SAA were included in the model simultaneously, the OR was attenuated and was no longer significant for CRP, but was still significant for SAA (data not shown). This may not contradict previous reports that suggest overlapping of the roles of the two markers and delineation of SAA as the more sensitive parameter (13,14).

The observed association did not differ for stratification by smoking status (never/past + current) for SAA; however, for CRP, the association was clearer among never smokers [2.50 (1.13–5.53)] compared with past and current smokers [1.15 (0.56–2.33)]. Using the World Health Organization category to adjust BMI did not alter the results essentially. When the interactions between each covariate in the model and CRP and SAA status were tested, no significant interaction was observed.

When all analyses were repeated in only those who were *H.pylori* positive (seropositive for IgG antibody and/or CagA), the associations were slightly attenuated, although they did not differ essentially; the adjusted ORs (95% CIs) for developing total gastric cancer were 1.72 (1.07–2.78, $P = 0.03$) for CRP-positive status and 1.82 (1.13–2.94, $P = 0.01$) for SAA-positive status, respectively.

Discussion

In this study, the overall distributions of CRP and SAA were not apparently associated with the development of gastric cancer. However, when subjects were divided on the basis of dichotomous categorization of positive versus negative, an increased risk was observed for positive subjects. The association was statistically significant even after adjustment for *H.pylori* infection, CagA status, atrophy and lifestyle factors. Elevated levels of CRP and SAA reflect a generalized host reaction that is either localized or systematic with regard to the initial event. Mechanisms of inflammation-associated tumor development are well described. These include stimulation of cellular proliferation (e.g. in cellular proto-oncogenes, DNA and cellular repair), inhibition of apoptosis, cellular adhesion, stimulation of angiogenesis and cellular transformation (1). In our data set, under the conditions that most subjects were infected with *H.pylori*, only those who reacted strongly to inflammation or had a high host immune response, as reflected by extremely elevated plasma levels of CRP and SAA, showed an elevated risk of developing the malignancy. The proportions of those who were categorized as positive were small; therefore, the findings should be interpreted with caution. However, this may be one of the explanations for why only a small proportion of *H.pylori*-infected subjects develop clinical consequences. CRP and SAA were useful markers to detect these high-risk groups.

Several clinical studies have shown that, compared with controls, gastric cancer patients have elevated CRP levels (24–26). Previous studies have even revealed that CRP has an impact on gastric cancer prognosis (24,27). It has been observed in previous studies that the SAA level increases in patients with stomach, lung, renal, colorectal, breast and other forms of cancers (28–35). With regard to gastric cancer, Chan *et al.* (28) demonstrated that patients with gastric cancer have higher SAA concentrations than do patients with gastric ulcers and healthy subjects and that levels of SAA correlate with tumor status, prognosis and recurrence. In our study, the average duration between blood donation and cancer diagnosis among cases was 5.4 years. When subjects who developed gastric cancer within 2 years of blood donation and their matched controls were excluded, the observed associations were strengthened; the adjusted ORs (95% CIs) for the association between development of gastric cancer and CRP and SAA positivity were 2.25 (1.31–3.85, $P = 0.003$) and 2.29 (1.32–3.95, $P = 0.003$), respectively. Furthermore, when subjects were stratified by the median duration between blood donation and

diagnosis (5.12 years), the adjusted ORs (95% CIs) for the association between development of gastric cancer CRP and SAA positivity within 5.12 years were 1.38 (0.69–2.73, $P = 0.36$) and 1.59 (0.82–3.09, $P = 0.17$), respectively. The values for CRP and SAA diagnosed after 5.12 years were 2.42 (1.23–4.77, $P = 0.01$) and 2.25 (1.12–4.52, $P = 0.02$), respectively. Therefore, our findings cannot be explained by the effect of preclinical samples among cases. Rather, our findings suggest that CRP and SAA may be useful markers for predicting the malignancy.

In our study, *H.pylori* seropositivity, *H.pylori* titer and atrophy were not distributed differently according to CRP status. Surprisingly, *H.pylori* seropositivity and atrophy were more frequent, and higher *H.pylori* titer was observed among SAA-negative subjects than among SAA-positive subjects. When the values were compared on the basis of tumor location, CRP did not show any difference; mean value (SE) was 0.09 (0.16) for cardia and 0.15 (0.05) for non-cardia cancer, respectively ($P = 0.75$). The value for SAA was 6.77 (1.87) for cardia, which was higher than that for non-cardia, 5.12 (0.62) ($P = 0.03$). High SAA level with an upper tumor site compared with a middle or a lower site was also observed by Chan *et al.* (28). Furthermore, the largest OR was observed for cardia cancer for both CRP and SAA. It is well known that *H.pylori* infection is related to non-cardia gastric cancer. As the majority of our subjects were infected with *H.pylori*, we were unable to show the results among *H.pylori*-seronegative subjects. Therefore, we cannot clarify whether the observed phenomenon was independent of *H.pylori*. We can state only that the observed elevated risk of gastric cancer with high levels of CRP and SAA is probably a phenomenon that cannot be totally explained by *H.pylori*; this conclusion is in line with that of previous studies (26,36). Comparing 153 preoperative gastric cancer patients with 19 healthy subjects, Tsavaris *et al.* (26) observed high serum levels of CRP, ceruloplasmin and a1-acid glycoprotein in cancer patients; however, among cancer patients, CRP level did not differ by status of *H.pylori* infection. Also, Delanghe *et al.* (36) showed that neither SAA nor other acute-phase proteins, including CRP, correlated with *Chlamydia pneumoniae* IgG, *H.pylori* IgG and IgA and cytomegalovirus IgG. On the other hand, the reason for the large OR observed in the cardia for both CRP and SAA positivity is unknown. One recent study reported that plasma CRP levels were associated with high BMI and other indicators of obesity (37). On the other hand, some studies, but not all, have proposed that elevated body weight may increase the risk of gastroesophageal reflux, which has been associated with adenocarcinomas of the gastroesophageal junction (38). Therefore, it is possible that elevated CRP and SAA were strongly associated with cardia cancer because of BMI status. However, in our data set, BMI did not differ by either CRP status or SAA status. The observed high OR in cardia cancer may be due to factors other than BMI or may be a mere chance finding.

On the basis of self-reported information, we adjusted for any condition that might alter the plasma levels of CRP or SAA. When these subjects were deleted (61 pairs; corresponds to 12% of total subjects), the overall findings did not change essentially, except when CRP values were divided into quartiles; the P for trend then became not significant ($P = 0.44$). Alternatively, when subjects with an extremely high level of CRP (>0.5 mg/dl) or SAA (>16.5 μg/ml) were excluded (55 pairs; corresponds to 11% of total subjects), the observed ORs became slightly higher, although the overall findings did not change essentially. To ensure the generalizability of findings and statistical power, we retained these subjects in the analyses.

Our study has several limitations. First, among 97 644 eligible subjects of the JPHC Study cohort, 36 745 (38%) men and women participated in the survey and provided blood samples. As reported previously, compared with non-participants, participants in the health checkup survey, especially women, had a different socioeconomic status and a favorable lifestyle profile, such as less smoking and alcohol consumption, greater participation in physical exercise and greater consumption of fruits or green vegetables (39). These findings mean that caution is needed in generalizing or interpreting the results in this report. Second, because of the relatively small sample size, further studies are needed to test our findings in analyses conducted by tumor location and histologic subtype.

The advantage of this study is its population-based prospective design and analysis of prediagnosed blood samples. Also, detailed information including *H.pylori* infection, CagA status, atrophy and environmental factors contributed to the detection of the relationships independent of these factors. Other strengths include negligible loss to follow-up and the satisfactory quality of our cancer registry system during the study period.

In conclusion, the overall distribution of CRP and SAA was not apparently associated with the development of gastric cancer. However, it was suggested that those who react strongly to inflammation or who have high host immune response, as reflected by extremely elevated plasma levels of CRP and SAA, were at high risk to develop gastric cancer.

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Elevated risk of colorectal adenoma with *Helicobacter pylori*-related chronic gastritis: a population-based case-control study

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This study investigated correlations between *Helicobacter pylori* infection or chronic atrophic gastritis (CAG) and risk of colorectal adenoma in a population-based case-control study. Subjects comprised asymptomatic, middle-aged, male Japanese factory workers who participated in an annual health check-up program, including cancer screening with colonoscopy. We selected 239 colorectal adenoma cases based on histological evaluation and 239 age-matched adenoma-free controls, and evaluated colorectal adenoma risk according to stage of *H. pylori*-related chronic gastritis as determined by serum tests for *H. pylori* antibody titer and pepsinogen. Subjects with colorectal adenoma were more likely to be smokers and have hypercholesterolemia. *H. pylori* infection was a risk factor for adenoma as a whole (crude odds ratio [OR]: 2.26, 95% confidence interval [CI]: 1.44–3.55). Analysis of distal adenoma cases showed that adenoma risk was significantly increased in the presence of *H. pylori* infection, but there was no further increase in risk with CAG. In contrast, proximal adenoma risk increased stepwise with the presence and progression of *H. pylori*-related chronic gastritis and showed a maximal and significant increase with CAG (crude OR: 4.51, 95% CI: 1.43–14.2). Subjects with more extensive and severe gastritis showed still higher risk not only for proximal but also for distal adenoma. *H. pylori*-related chronic gastritis is likely to be involved in the development of colorectal neoplasms, and its progression appears to increase the risk, particularly for proximal adenomas. Knowing the *H. pylori*-related chronic gastritis stage will probably be useful for evaluation of risk for colorectal neoplasia.

Helicobacter pylori infection induces chronic inflammation in the stomach mucosa of both humans and animals, and *H. pylori*-related chronic gastritis is deeply involved in the development of gastric neoplasms, such as adenoma or cancer.[1] *H. pylori* infection is now widely accepted as a major driving force in the progression of a series of carcinogenic cascades representing the gastritis-atrophy-metaplasia-dysplasia-cancer sequence.[2] Our previous seroepidemiological study clearly demonstrated a positive correlation between progression of chronic gastritis and risk of gastric cancer. Subjects with extensive chronic atrophic gastritis (CAG), as determined by serum pepsinogen (PG) levels, showed an annual cancer incidence rate of 0.24%.[3]

Recently, promotion of tumor development by *H. pylori* infection in extragastric target organs has been reported.[4] In addition, some clinical and epidemiological studies have revealed close correlations between incidence rates of gastric and colorectal mucosal neoplasms.[5,6] Furthermore, previous studies have suggested that the most common second primary site of synchronous and metachronous cancer in cases of gastric cancer is the colorectum.[7,8] In Japan, areas with high age-adjusted mortality rates from stomach cancer among the 47 municipal districts, such as Wakayama Prefecture (51.1/100,000 person-years), have also reported high rates of colorectal cancer mortality (38.4/100,000 person-years).[9] Progressive chronic gastritis induced by persistent *H. pylori* infection leads to extensive glandular atrophy and reduced acid secretion, which induces hypergastrinemia, a putative trophic factor for large bowel mucosa,[10] and it alters the gastrointestinal microenvironment composed of bacterial flora,[11] and thus may contribute to colorectal

Key words: colorectal adenoma, pepsinogen, chronic atrophic gastritis, case-control study, cancer risk

Abbreviations: BMI: body mass index; CAG: chronic atrophic gastritis; CI: confidence interval; CIMP+: CpG island methylator phenotype; CIN: chromosomal instability; ELISA: enzyme-linked immunosorbent assay; IgG: immunoglobulin G; MSI+: microsatellite instability; OR: odds ratio; PG: pepsinogen; SD: standard deviation; TC: total cholesterol; TG: triglyceride

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carcinogenesis. On the basis of these findings, we investigated the possibility that gastric cancer/adenoma and colorectal cancer/adenoma have common risk factors of *H. pylori* infection and/or its end result, CAG. The aim of our study was to examine etiological links among precancerous colorectal lesions, adenomas and *H. pylori* infection or CAG.

Material and Methods

Subjects

Between April 1996 and March 2004, a total of 4,655 middle-aged male factory workers (mean age ± standard deviation [SD]: 49.5 ± 4.6 years; range: 40–59 years) underwent annual health check-ups at a workplace in Wakayama City, located in the southwestern part of the main island of Japan. All patients were inhabitants from the Wakayama area. This type of screening program is common in various workplaces throughout Japan to detect incident diseases in early stages. Thus, subjects were symptom-free and workers with symptoms requiring prompt medical care were excluded from the study. As a result, all subjects in this study could be considered to represent healthy Japanese individuals.

As part of the screening program, study subjects underwent a series of screening tests and procedures: an interview to ascertain general state of health, physical examination, chest radiography, electrocardiography, blood laboratory testing, urinalysis and barium X-ray. In addition, subjects could select to undergo screening for colorectal cancer using their preferred method among fecal occult blood testing (FOBT), barium enema or colonoscopy. During the study period, a total of 1,605 middle-aged men underwent colonoscopy for cancer screening. Subjects with a previous history of colorectal neoplasia or inflammatory bowel disease were excluded from the study. In addition, subjects who underwent colonoscopy because of positive FOBT or a positive finding on barium enema were also excluded. As a result, a total of 1,019 subjects were analyzed for the study. When each case of colorectal adenoma was identified, an age-matched control (within 3 years) was randomly selected from among the participants of the health check-up program who were confirmed to be colorectal neoplasm-free.

Questionnaire

Information about baseline characteristics (age, height, weight, sociodemographic characteristics, personal medical history, family history, smoking and alcohol consumption) was obtained from the questionnaire completed at the time of the aforementioned interview.

Evaluation of CAG and *H. pylori* infection

Aliquots of separated sera from blood samples collected as routine laboratory tests for the general health check-up were stored below −20°C until measurement of serum levels of *H. pylori* immunoglobulin (Ig) G antibody titer and serum PG. *H. pylori* IgG antibody titers were measured using an enzyme-linked immunosorbent assay (ELISA) (MBL, Nagoya, Japan). Antibody titers >50 U/ml were classified as indicating *H. pylori* infection. The sensitivity and specificity of the ELISA used in our study were 93.5% and 92.5%, respectively.[12] Serum PG levels were measured using PGI/PGII RIA-Bead Kits (Dainabbot, Tokyo, Japan), which use a modified radioimmunoassay method that we previously established.[13] Subjects with extensive CAG were diagnosed on the basis of the previously described PG test-positive criteria (PG I $\leq$ 70 ng/ml and PG I/II $\leq$ 3.0).[14,15] These criteria have 70.5% sensitivity and 97% specificity.[14] Subjects who had been prescribed medications before examination that might affect gastrointestinal function, such as proton pump inhibitors, H2 blockers or nonsteroidal anti-inflammatory drugs, as well as subjects who had a previous history of gastric resection, *H. pylori* eradication therapy or renal failure, were excluded from analysis of PG test results.

In our study, all but four CAG cases diagnosed by the above PG-test positive criteria were *H. pylori*-antibody positive. Endoscopic examination of these four *H. pylori*-negative CAG cases (one control and three adenoma cases) revealed extensive metaplastic gastritis involving both antrum and corpus. Thus, the negative result for *H. pylori* antibody is considered to reflect a spontaneous eradication of the bacteria, an end result of the progression of *H. pylori*-related CAG. Furthermore, the prevalence of autoimmune gastritis is extremely low in Japan; the incidence rate is reported to be 0.6/100,000 person-years.[16] Thus, the possibility of autoimmune gastritis in the analyzed CAG cases including these four *H. pylori*-negative cases is considered to be negligible.

Screening for colorectal neoplasia

Subjects who selected colonoscopy for colorectal cancer screening underwent a full colonoscopic examination with adequate bowel preparation. A colonoscope (CF 240 I, Olympus, Tokyo, Japan) was inserted to the cecum, except in cases with advanced adenocarcinoma. The adenoma cases were classified into three groups according to the location of the detected polypoid lesion: proximal (cecum, ascending colon, hepatic flexure and transverse colon), distal (splenic flexure, descending colon, sigmoid colon and rectum) and bilateral (lesions located in both sides). All polypoid lesions found during colonoscopy were biopsied, immediately fixed in 10% formalin and embedded in paraffin. Tissue sections were stained with hematoxylin-eosin and examined under light microscopy. Routine histological evaluation was performed by staff pathologists.

The retrospective analysis of the clinical data in our study was approved by the ethics committee of Wakayama Medical University. Informed consent for the use of the clinical data from the health check-up was obtained from the screened subjects at the time of their first screening.

Table 1. Baseline characteristics of study subjects

	Control $n = 239$	Case $n = 239$	p^1	Proximal $n = 38$	p^1	Bilateral $n = 78$	p^1	Distal $n = 123$	p^1
Age (years)									
Mean (SD)	49.4 (4.3)	49.9 (3.9)	0.21	49.7 (3.9)	0.71	49.6 (3.8)	0.65	50.1 (4.0)	0.14
BMI (kg/m^2)									
Mean (SD)	23.4 (2.9)	23.7 (2.8)	0.16	23.7 (2.5)	0.53	24.0 (2.7)	0.08	23.6 (3.0)	0.50
Current smoker (–)/(+)	103/136	83/156	0.08	18/20	0.75	18/60	0.002	47/76	0.43
Alcohol use (–)/(+)[2]	73/166	62/177	0.31	9/29	0.5	18/60	0.26	35/88	0.77
TC (mg/dl)									
Mean (SD)	204.5 (31.3)	210.0 (34.2)	0.07	205.1 (39.3)	0.92	207.6 (32.8)	0.44	212.9 (33.3)	0.02
TG (mg/dl)									
Mean (SD)	167.7 (130.3)	183.1 (137.6)	0.21	175.0 (94.4)	0.74	205.3 (155.4)	0.06	171.5 (136.0)	0.79
***H. pylori* IgG (U/ml)**									
Mean (SD)	288.0 (479.7)	352.0 (462.6)	0.14	354.7 (463.4)	0.42	316.4 (438.8)	0.63	373.8 (479.1)	0.11
PG I (ng/ml)									
Mean (SD)	58.7 (29.5)	58.1 (25.9)	0.81	54.5 (23.3)	0.4	59.2 (23.6)	0.89	58.6 (28.0)	0.96
PG II (ng/ml)									
Mean (SD)	15.8 (10.0)	18.5 (11.0)	0.006	18.1 (8.7)	0.18	18.0 (10.7)	0.10	18.9 (11.8)	0.02
PG I/II									
Mean (SD)	4.4 (2.2)	3.7 (1.8)	<0.001	3.3 (1.7)	0.003	3.9 (1.9)	0.03	3.6 (1.8)	<0.001
Clinicopathological features									
Size									
The proportion of the cases with adenoma <10 mm (%)				177/239 (74.1)		32/38 (85)		49/78 (62.7)	96/123 (77.9)
The proportion of the cases with adenoma ≥10 mm (%)				62/239 (25.9)		6/38 (15)		29/78 (37.3)[3]	27/123 (22.1)
Number									
The proportion of the cases with a single adenoma (%)				120/239 (50.2)		31/38 (81.6)		0/78 (0)	89/123 (72.3)
The proportion of the cases with two or more adenoma (%)				119/239 (49.8)		7/38 (18.4)		78/78 (100)	34/123 (27.7)
Histopathology									
The proportion of the cases with tubular adenoma (%)				228/239 (95.4)		36/38 (95)		76/78 (98)	116/123 (94.1)
The proportion of the cases with tubulovillous, villous adenoma (%)				11/239 (4.6)		2/38 (5)		2/78 (2)	7/123 (5.9)
The grade of dysplasia									
The proportion of the cases with mild or moderate adenoma (%)				226/239 (94.6)		35/38 (92.1)		73/78 (93.6)	118/123 (95.9)
The proportion of the cases with sevear adenoma (%)				13/239 (5.4)		3/38 (7.9)		5/78 (6.4)	5/123 (4.1)

[1]Two-sided p-values for the difference between cases and controls were based on the χ^2 test and t test. [2]Drinking alcohol at least once a week for the past 5 years. [3]$p < 0.05$: vs proximal based on χ^2 test.
Abbreviations: BMI, body mass index; TC, total cholesterol; TG, triglycerides; PG, pepsinogen; CAG, chronic atrophic gastritis; SD, standard deviation.

Statistical analysis

Data were analyzed using SPSS version 11.0 (Chicago, IL) and STATA (College Station, TX). Data for continuous variables were expressed as mean ± SD, and the differences were tested for significance using t tests for comparison of two groups and analysis of variance (ANOVA) for comparison among multiple groups. Categorical variables were compared using the chi-squared test. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to describe associations. ORs with corresponding 95% CIs were obtained by conditional logistic regression analysis. Trend tests were assessed using an ordinal score for each categorical variable. All two-sided p-values less than 5% were considered statistically significant.

Results

In our study, 239 middle-aged men with colorectal adenoma were identified. Although four cases of adenocarcinoma were also detected during the study period, they were excluded from the study because of the small number. The same number of age-matched adenoma-free controls was randomly selected from among asymptomatic middle-aged factory workers. Baseline characteristics of colorectal adenoma cases