British Journal of Nutrition

of 1231 subjects who were aged 40–69 years and living in four municipalities of the Yokote Public Health Center District of Akita Prefecture were selected to participate in the randomised clinical trial. After the first year of participants' recruitment in 1995, β-carotene supplementation was reported to have potential harmful effects for individuals at high risk for lung cancer[12,13], and the study protocol was modified by removing subjects who were using β-carotene and stopping recruitment of new subjects in three municipalities[10]. The primary endpoint of the trial was changed from a 10-year accumulated incidence of gastric cancer to 5-year changes of the serum levels of pepsinogen (PG) and other biomarkers[10]. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving patients were approved by the ethics committee of the National Cancer Center and the Hiraka General Hospital. Written informed consents were obtained from all individuals willing to participant and those remaining in the study. Finally, 120 and 124 subjects in the low-dosage and high-dosage groups of vitamin C supplementation, respectively, completed the 5-year study (Fig. 1). The details of the study rationale, design, methodology and protocol amendment have been described previously[10,11].

Eligible subjects were diagnosed with chronic atrophic gastritis by the cut-off value of PGI <70 ng/ml and a ratio of PGI:II of $<3{\cdot}0$, of which the sensitivity was 80% and specificity was 70% as reported[14]. Miki[15] reported that the values measured by the same kit showed a good correlation (correlation coefficient 0·983 for PGI, 0·991 for PGII and 0·935 for PGI:II) with those measured by RIA (PGI/PGII RIA-BEAD; Dinabot Company Limited), in which a sensitivity of 70·5% and a specificity of 97·0% for atrophic gastritis, compared with histology, have been reported[16]. Selection criteria were no history of gastric cancer or related surgery; no history of cirrhosis, liver cancer or other cancer within the last 5 years; no abnormal liver function; no use of diet supplements containing β-carotene or vitamin C; and no expectation of moving outside the study area within 1 year.

Participant follow-up and dietary intake assessment

Participants were asked to visit the community centres every 3 months where their clinical symptoms and side effects from vitamin C supplementation were assessed, compliance was checked based on the number of unconsumed capsules, and capsules for further use were dispensed[10,11]. Compliance averaged 92·6 and 92·2% in the low- and high-dosage groups,

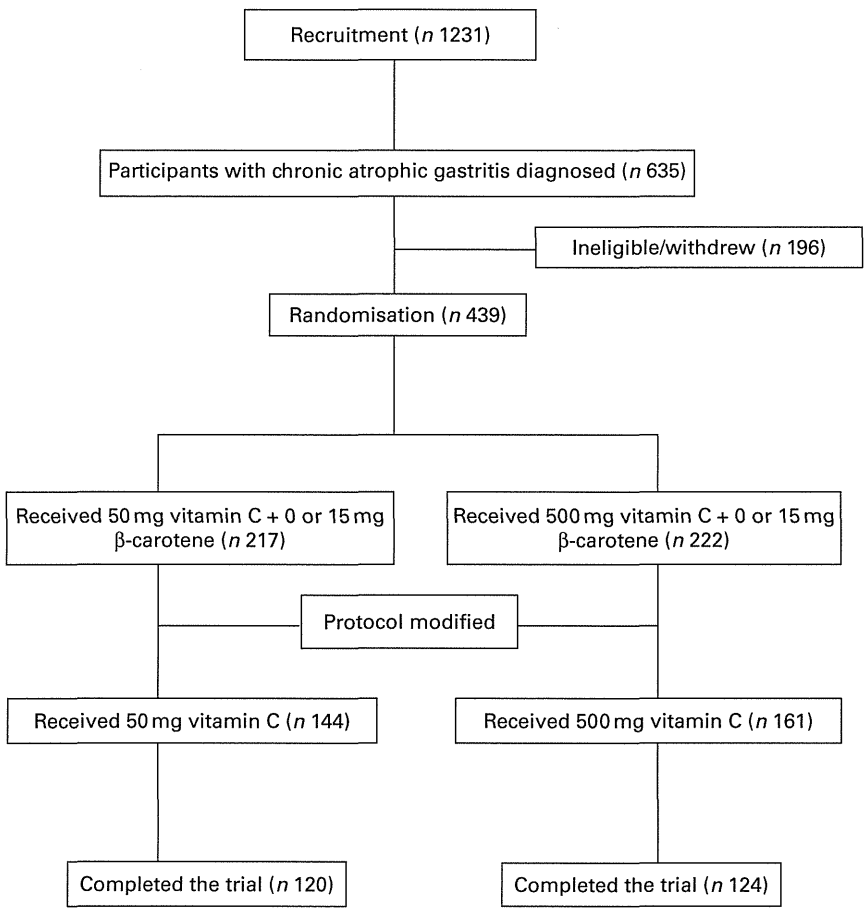

Fig. 1. Flow chart of participant recruitment before and after the protocol amendment and of participants at the 5-year follow-up.

respectively[17]. A validated 138-item FFQ was used to assess dietary intake, for which participants were asked how often they consumed individual food items and to estimate the representative size of their portions relative to the size of a standard portion. Daily intake of vitamin C and other nutrients were calculated by using the fifth revised and enlarged edition of the Standard Tables of Food Composition in Japan[18]. The details of the FFQ have been described in a previous report[11,17].

Biochemical analysis

Fasting blood samples were collected at baseline and after 5 years and analysed for serum ascorbic acid levels, CRP and SAA. The subjects were asked not to eat or drink anything except water after 21.00 hours on the day before blood sampling. The serum was sampled between 07.00 and 10.00 hours. All samples were stored at −70 to −85°C and were analysed simultaneously after completion of the 5-year follow-up. All assays were conducted by persons who were blinded as to the intervention assignment and the questionnaire data.

Serum for ascorbic acid measurement was stabilised by the addition of metaphosphoric acid, and serum ascorbic acid concentration was measured fluorimetrically (iodine oxidation and condensation with 1,2-phenylenediamine). CRP and SAA concentrations were determined by the latex agglutination nephelometric immunoassay test (LZ test 'Eiken' CRP-HG and LZ test 'Eiken' SAA, respectively; Eiken Kagaku Company Limited). IgG antibodies to *H. pylori* were measured with a direct ELISA kit (E Plate 'Eiken' *H. pylori* antibody; Eiken Kagaku Company Limited). Levels of IgG were categorised as seropositive and seronegative for *H. pylori* according to the selected cut-off value (492 nm)[19].

Statistical analysis

We followed the intent-to-treat analysis, which included all subjects remaining in the study after the protocol was modified. The per-protocol analysis included subjects who completed the study to the 5-year follow-up. Baseline comparisons between the low- and high-dosage groups and the dropout group as the control were examined by one-way ANOVA for continuous variables and by the χ^2 test for categorical variables. Differences of values within the low- and high-dosage groups were tested by the paired t test for continuous variables and by the one-sample z test for proportions.

CRP was categorised into positive and negative groups by using a cut-off point of 1·8 mg/l, while SAA was grouped as positive or negative based on a cut-off point of 8·0 μg/ml[3]. Subjects' status on combined biomarkers of CRP and SAA was determined by the defined positive and negative statuses of CRP and SAA. Log transformation was done for dietary intake of vitamin C, serum CRP and SAA, and *H. pylori* titre when conducting the comparisons between the two dosage groups; and data are presented as geometric means with their standard errors. The difference between the two dosage groups for changes in CRP and SAA at the end of the 5-year follow-up compared with baseline was calculated by using the geometric means, respectively.

Adjusted analysis of the means of serum CRP and SAA for covariates was performed by one-way ANOVA. Results were adjusted for age (continuous), sex, dietary intake of vitamin C (quartile), alcohol consumption (never or occasional, regular), smoking status (never, ever), BMI (<25, ≥ 25 kg/m^2), *H. pylori* status (no, yes) and menopausal status (no, yes, for women). Stratified analysis was performed for age groups, alcohol consumption, smoking status, BMI and menopausal status. P values less than 0·05 in two-tailed tests were considered as significant, and all statistical analyses were performed using SAS version 9.1 (SAS Institute).

Results

The baseline characteristics of the trial participants are shown in Table 1. Subjects in the low-dosage group were older than those in the high-dosage group. There were more CRP-positive subjects in the high-dosage group than in the low-dosage group both in the intent-to-treat and per-protocol analyses (borderline significance). *H. pylori* titres were higher in the high-dosage group than in the low-dosage group, with a significant difference in the per-protocol analysis.

At the 5-year follow-up, serum ascorbic acid was higher in the high-dosage group (increased 0·37 μg/l) compared with the low-dosage group (increased 0·10 μg/l from baseline, $P<0·001$) (Table 2). Correlation of the log-transformed CRP and SAA in all participants at the 5-year follow-up was 0·541 ($P<0·001$). A slight increase in the low-dose group and a decrease in the high-dose group both in CRP and SAA levels were observed at the 5-year follow-up; thus the absolute 0·07 mg/l reductions in CRP and the 0·31 μg/ml reduction in SAA were in the high-dose group compared with those in the low-dose group, if taking consideration of the baseline values. However, there were no significant differences for CRP between the low- and high-dosage groups (0·39 (95% CI 0·04, 4·19) mg/l and 0·38 (95% CI 0·03, 4·31) mg/l, respectively; $P=0·63$) or for SAA between the low- and high-dosage groups (3·94 (95% CI 1·04, 14·84) μg/ml and 3·85 (95% CI 0·99, 14·92) μg/ml, respectively; $P=0·61$) (Table 2). CRP status changed from positive to negative for 60% (six out of ten) of the low-dosage group and 68·4% (thirteen out of nineteen) of the high-dosage group between baseline and the 5-year follow-up ($P=0·33$), while SAA status for 57·1% (eight out of fourteen) in the low-dosage group and 70·0% (seven out of ten) in the high-dosage group of SAA-positive participants changed from positive to negative ($P=0·27$). The combined positive and negative statuses for CRP and SAA were also not significantly different between the two groups at the 5-year follow-up (47·4% (nine out of nineteen) *v.* 59·1% (thirteen out of twenty-two); $P=0·23$). When we deleted two outliers that were both CRP- and SAA-positive at baseline, similar null results for CRP and SAA were observed, respectively, between the two dosage groups at the 5-year follow-up.

Table 1. Baseline characteristics of the participants in the trial
(Mean values and standard deviations or standard errors; number of participants and percentages)

	Intent-to-treat					Per-protocol				
	Low-dosage vitamin C, 50 mg (*n* 144)		High-dosage vitamin C, 500 mg (*n* 161)			Low-dosage vitamin C, 50 mg (*n* 120)		High-dosage vitamin C, 500 mg (*n* 124)		
	n	%	*n*	%	*P**	*n*	%	*n*	%	*P**
Age (years)					0·01					0·02
Mean	58·56		56·55			58·67		56·29		
SD	6·64		8·74			6·53		8·66		
BMI (kg/m^2)					0·66					0·52
Mean	23·38		23·18			23·42		23·23		
SD	2·92		2·69			2·86		2·65		
Men	54	37·5	58	36·0	0·79	41	34·2	45	36·3	0·73
Current smoking	18	12·5	26	16·2	0·65	12	10·0	19	15·3	0·38
Alcohol consumption	62	43·1	69	42·9	0·99	53	42·2	57	46·0	0·69
Serum ascorbic acid (μg/l)	1·37	0·35	1·35	0·37	0·96	1·38	0·32	1·35	0·37	0·51
Dietary vitamin C (μg/l)					0·65					0·41
Mean	121·03		120·25			123·62		123·52		
SE	1·06		1·06			1·06		1·06		
CRP (mg/l)†					0·16					0·77
Mean	0·35		0·43			0·35		0·41		
SE	1·11		1·13			1·12		1·14		
CRP positive†	11	7·6	24	14·9	0·05	10	8·3	19	15·3	0·07
SAA (μg/ml)†					0·26					0·29
Mean	3·82		4·29			3·87		4·09		
SE	1·06		1·08			1·07		1·09		
SAA positive†	16	11·1	16	9·9	0·74	14	11·7	10	8·1	0·35
Helicobacter pylori titre (RU/ml)					0·15					0·01
Mean	59·19		68·73			57·13		73·73		
SE	1·07		1·07			1·08		1·07		
H. pylori positive	140	97·2	157	97·5	0·87	116	96·7	122	98·4	0·39
PGI (ng/ml)					0·83					0·65
Mean	38·38		39·03			38·35		39·8		
SD	17·06		16·43			17·2		16·35		
PGII (ng/ml)					0·15					0·11
Mean	19·62		20·60			19·46		20·79		
SD	7·14		7·34			7·22		7·34		
PGI:II					0·20					0·24
Mean	1·95		1·89			1·97		1·92		
SD	0·63		0·61			0·62		0·59		

CRP, C-reactive protein; SAA, serum amyloid component A; RU, relevant unit; PG, pepsinogen.
* By one-way ANOVA test or χ^2 test.
† 117 subjects in the per-protocol analysis were available in the low- and high-dosage groups, respectively.

Stratified analysis showed that there were no significant differences in the decrease in CRP or SAA levels between the two dosage groups by age categories (40s, 50s and 60s), sex, smoking or alcohol consumption. Similar results were observed after adjusting for sex, dietary intake of vitamin C (quartile), *H. pylori* titre, smoking status, alcohol consumption and BMI (data not shown).

Discussion

We did not observe any significant reduction of CRP or SAA levels in the low- or high-dosage groups after 5 years of ascorbic acid supplement use, although serum ascorbic acid concentration was higher in the high-dosage group than in the low-dosage group. We also did not observe any significant differences between the two groups in age, sex, smoking, alcohol consumption or body weight status.

The CRP and SAA levels in the present study were similar to those reported in other studies[3,20]. In the present study, based on cut-off points of 1·8 mg/l for CRP and 8·0 μg/ml for SAA, there were small numbers of CRP- or SAA-positive participants and there was no significant difference for either between the two dosage groups at baseline, respectively. We also applied other cut-off points for CRP- and SAA-positive status such as a CRP of 10 mg/l[21] or by areas under the received curve[22]. By these criteria, the numbers of CRP- or SAA-positive participants remained similar and no significant differences existed between the two dosage groups. Nevertheless, the small number of CRP- and SAA-positive participants at baseline made it difficult to evaluate changes in CRP and/or SAA status at follow-up. It might be possible that CRP and SAA were not highly sensitive markers for measuring chronic infection status, which contributed to the null outcome in the present study. On the other hand, the 500 mg/d supplement in the present study might not be sufficient to control chronic gastric infection, although cancer chemoprevention trials with more than 500 mg/d of vitamin C

Table 2. Comparisons of serum ascorbic acid and inflammatory biomarkers between baseline and the 5-year follow-up (Mean values and standard deviations or standard errors)

	Low-dosage vitamin C, 50 mg (*n* 117)					High-dosage vitamin C, 500 mg (*n* 117)					
	Baseline		5 years			Baseline		5 years			
	Mean	SE	Mean	SE	*P**	Mean	SE	Mean	SE	*P**	*P*†
Serum ascorbic acid (μg/l)					<0·01					<0·001	<0·001
Mean	1·38		1·49			1·35		1·73			
SD	0·32		0·29			0·37		0·46			
Dietary vitamin C (μg/l)	123·62	1·06	121·14	1·06	0·79	123·52	1·06	123·11	1·06	0·88	0·78
CRP (mg/l)	0·35	1·12	0·39	1·12	0·35	0·41	1·14	0·38	1·12	0·64	0·63‡
SAA (μg/ml)	3·87	1·07	3·94	1·06	0·88	4·09	1·09	3·85	1·07	0·57	0·61‡

CRP, C-reactive protein; SAA, serum amyloid component A.
* By paired *t* test.
† By one-way ANOVA test for the difference between the two dose groups at the 5-year follow-up.
‡ Adjusted for age, sex, BMI, smoking status, alcohol consumption, dietary vitamin C, *Helicobacter pylori* status and baseline level of CRP or SAA.

supplementation have not shown consistent results on the beneficial effects[23,24].

Human gastric carcinogenesis is a multistep and multifactorial process, with the initial stages of gastritis and atrophy linked to excessive salt intake and *H. pylori* infection[17,25]. *H. pylori* eradication can prevent the progression of precancerous gastric lesions and probably reduce the incidence of gastric cancer in those without advance lesions[26]. In the present study, CRP and SAA were not significantly reduced and the positive proportions of *H. pylori* were consistently higher (≥92%) after 5 years of follow-up in both the low- and high-dosage groups[17]. It was possible that in the achlorhydric stomach, *H. pylori* infection might disappear, although the antibodies in the serum might maintain a longer time. Nevertheless, *H. pylori* infection potentially modulates the effects of vitamin C or vice versa[9]. Without eradicating the infection, ascorbic acid supplementation for participants with atrophic gastritis might have fewer effects on CRP/SAA control. However, studies on changes in CRP after *H. pylori* eradication are contradictory. Some studies have reported a significant reduction of CRP levels in subjects after *H. pylori* eradication by antibiotics[27] or vitamin C supplementation[28], while others have shown no significant reduction of CRP by anti-inflammatory or antibiotic treatment[20,29,30]. A Colombian study in gastritis patients, applying a 2-week anti-*H. pylori* treatment and/or a 6-year antioxidant supplement, showed that acute inflammation disappeared soon after the *H. pylori* treatment, while chronic inflammation responded at a slower pace, and the antioxidant effect was transient and disappeared after the 6 years of follow-up, while the anti-*H. pylori* treatment effect persisted for as long as patients remained free of *H. pylori*[23]. Also, subjects with non-metaplastic multifocal atrophic gastritis had the steepest declines if they cleared the bacteria, but had the sharpest increases if they did not[23]. The present study results appear to support the finding that ascorbic acid supplementation does not have much beneficial effect on chronic gastric infections, particularly without assigning the anti-*H. pylori* treatment.

There are several limitations in the present study. The most critical one is that we did not have a placebo group for comparison with the 50 and 500 mg dosage groups[31]. However, the mean dietary intakes of vitamin C were 151·95 (SD 111·98) μg/l and 147·93 (SD 99·81) μg/l for the high- and low-dose groups, respectively, and the low-dose supplementation group was similar to or within 1 SD of the estimated vitamin C intake level from foods. In the pilot study[32] for the present trial, there were no significant differences in serum vitamin C concentrations between the placebo (0 mg/d) and the low-dose groups at 1, 2 and 3 months of supplementation, respectively. Moreover, the purpose of the present study was to evaluate the effect of vitamin C supplementation (500 mg/d) compared with the normal level (the average consumption level of Japanese). Additionally, the similar mean dietary intake of vitamin C in the placebo group was seen in another trial[33]. Therefore, the low-dose vitamin C supplementation group (50 mg/d) in the present study could be regarded as the placebo group for interpretation[34]. Second, the initial sample size was considered with estimated differences in accumulated gastric cancer incidence between the two study groups in 10 years rather with the changes in these biomarkers of atrophic gastritis in 5 years[10]. For example, to detect the 0·15 μg/ml difference in SAA levels between the two dose groups at the 5-year follow-up, using the standard deviation in each group, 5% type I error and 20% type II error for estimation, 1030 subjects in each group are needed. The limited number of study subjects after changes in the initial study protocol had less statistical power for identifying the significance of CRP and SAA reductions between the two dosage groups. Third, IL-6 and other immunological factors are thought to be mediators that stimulate CRP production[5,28,35]; however, we could not evaluate the CRP reduction as modified by ascorbic acid by using these factors because the data were unavailable. Since we did not conduct endoscopy for gastritis participants, we therefore could not evaluate the progression or regression of gastric lesions after ascorbic acid supplementation at the 5-year follow-up[23,36,37]. Finally, since we only tested CRP and SAA two times, at baseline and the 5-year follow-up, any changes in their levels in the intervening time were not evaluated.

Some studies have reported that antioxidant supplementation, even at low doses, can have adverse effects on subjects at high risk for cancer or those with undiagnosed cancer[38,39].

It should be noted that some of the well-known beneficial effects of ascorbic acid administration are still only understood at the phenomenological level[40]. Currently, the Asia–Pacific guidelines on gastric cancer prevention do not recommend vitamin C supplementation for reducing the risk of gastric cancer[26].

In summary, we did not observe a significant reduction in CRP or SAA levels in atrophic gastritis participants with ascorbic acid supplementation of less than 500 mg/d at the 5-year follow-up. The present study suggests that ascorbic acid supplementation might not have much beneficial effect in individuals with chronic *H. pylori* infection. Further studies are needed in larger populations on the control of chronic infection and inflammation through ascorbic acid supplementation.

Acknowledgements

We wish to express our appreciation to the staff at the Hiraka General Hospital and the public health nurses at Sannai village office for their support and assistance with the study. This study was supported by Management Expenses Grants from the Government to the National Cancer Center and 3rd Term Comprehensive 10-Year Strategy for Cancer Control from the Ministry of Health, Labour and Welfare of Japan. Y. T. and S. T. designed the current research; Y. T. and S. T. initiated, modified and set the study protocols; Y. T., S. Sasaki, S. O. and S. Sasazuki implemented the study and collected the data; E. M. and S. Sasazuki analysed the data; E. M. and S. Sasazuki wrote the paper. All authors read and approved the final manuscript. The authors declare that there are no conflicts of interest.

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British Journal of Nutrition

JJCO Japanese Journal of Clinical Oncology

Jpn J Clin Oncol 2012;42(4)335–346
doi:10.1093/jjco/hys009
Advance Access Publication 27 February 2012

Public Health Report

Green Tea Consumption and Gastric Cancer Risk: An Evaluation Based on a Systematic Review of Epidemiologic Evidence Among the Japanese Population

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Received October 19, 2011; accepted January 24, 2012

Objective: Numerous *in vitro* and animal studies have shown that green tea has a protective effect against cancer. However, results from epidemiologic studies are conflicting. We evaluated the association between green tea consumption and risk for gastric cancer risk among the Japanese population based on a systematic review of epidemiologic evidence.
Methods: Original data were obtained from MEDLINE searches using PubMed or from searches of the *Ichushi* database, complemented with manual searches. Evaluation of associations was based on the strength of evidence and the magnitude of association, together with biologic plausibility.
Results: Eight cohort studies and three case–control studies were identified. Overall, we found no preventive effect on gastric cancer for green tea intake in cohort studies. However, a small, consistent risk reduction limited to women was observed, which was confirmed by pooling data of six cohort studies (hazard ratio = 0.79, 95% confidence interval 0.65–0.96 with ≥5 cups/day of green tea intake). Case–control studies consistently showed a weak inverse association between green tea intake and gastric cancer risk.
Conclusions: We conclude that green tea possibly decreases the risk of gastric cancer in women. However, epidemiologic evidence is still insufficient to demonstrate any association in men.

Key words: systematic review – epidemiology – green tea – gastric cancer – Japanese

BACKGROUND

Although the age-standardized mortality rate has been continuously declining, gastric cancer is still the second leading cause of cancer deaths among men and women in Japan (1). In addition to *Helicobacter pylori* infection or cigarette smoking, dietary factors are suggested to be associated with gastric carcinogenesis (2).

Numerous *in vitro* and animal studies have shown that green tea has a protective effect against cancer (3). These experimental studies have suggested that green tea polyphenols might have a protective effect against gastric cancer through its apoptosis-inducing, antimutagenic and antioxidant activities. In 1997, a review of the World Cancer Research Fund, based on the results of case–control studies and several animal models that showed a protective effect of green tea, stated that 'green tea possibly reduce the risk of stomach cancer' (4). Since then, results from cohort studies generally have not supported the findings from case–control studies, and the more recent 2007 report concluded that 'the evidence was so limited that no firm conclusion can be made' (5).

In Japan, green tea is one of the most commonly consumed beverages, and therefore, the effect of green tea on the risk for gastric cancer may be of particular concern. We reviewed epidemiologic studies of green tea consumption and gastric cancer risk among Japanese. This work was conducted as a systematic review of epidemiologic studies on lifestyle factors and cancer based on previous publications targeting Japanese (6).

METHODS

Research Review

Details of the evaluation method have been described previously (6). Briefly, original data for this review were identified through searches of the MEDLINE (PubMed) and *Ichushi (Japana Centra Revuo Medicina)* databases, complemented by manual searches of references from relevant articles where necessary. All epidemiologic studies on the association between green tea intake and gastric cancer incidence/mortality among the Japanese from 1950 (or 1983 for the *Ichushi* database) to June 2011 were identified using the search terms 'green tea', 'tea', 'gastric cancer', 'stomach cancer', 'cancer', 'cohort study', 'case-control study', 'Japan' and 'Japanese' as key words. In addition, we manually searched through references from relevant articles where necessary. Papers written in either English or Japanese were reviewed, and only studies on Japanese populations living in Japan were included. In the case of multiple publications of analyses of the same or overlapping data sets, only data from the largest or the most recent studies were included. The individual results were summarized in the tables separately as cohort or case–control studies. Pooled data of Japanese cohort studies, including some of the individual studies already listed, were also available through the review process. To better understand the results from individual studies and finally evaluate the evidence for green tea intake and gastric cancer risk in Japanese, findings from recent pooled analyses were also listed and considered in this report.

Evaluation of Strength of Association Between Green Tea Intake and Gastric Cancer Risk

The evaluation was made based on the magnitude of association and the strength of evidence. First, the former was assessed by classifying the relative risk (RR) in each study into the following four categories, while considering statistical significance (SS) or no statistical significance (NS), as strong (symbol ↓↓↓ or ↑↑↑), <0.5 or >2.0 (SS); moderate (symbol ↓↓ or ↑↑), either (i) <0.5 or >2.0 (NS), (ii) >1.5–2 (SS) or (iii) 0.5 to <0.67 (SS); weak (symbol ↓ or ↑), either (i) >1.5–2 (NS), (ii) 0.5 to <0.67 (NS) or (iii) 0.67–1.5 (SS); or no association (symbol –), 0.67–1.5 (NS). In cases where the frequency or amount of green tea intake had been separated into levels in a study, we used the RR derived from comparing the highest intake with the lowest. To consider the intermediate categories of intake, however, the *P* value for the trend was also considered when judging the statistical significance. After this process, the strength of evidence was evaluated in a manner similar to that used in the WHO/FAO Expert Consultation Report, where evidence was classified as convincing, probable, possible and insufficient (7). We assumed that biologic plausibility was based on evidence in experimental models, human studies and other relevant data.

MAIN FEATURES AND COMMENTS

Through the review process, we identified eight cohort studies (8–15), one pooled analysis of six cohort studies (16) (Table 1) and three case–control studies (17–19) (Table 2). Among cohort studies, the events followed were death in five studies (8,10,12,14,15) and incidence in the other three studies (9,11,13). Five studies showed the results for men and women separately (9,10–14), whereas three studies showed combined results only (8,13,15). The pooled analysis included four cohorts (9,10 and two cohorts in 11) listed in Table 1 and two other cohorts (20,21). For all case–control studies, the results were shown for men and women combined.

The summary of the magnitudes of association for the cohort study and the case–control study is presented in Tables 3 and 4, respectively. As shown in Table 3, among eight cohort studies, one study showed a weak positive association between green tea intake and gastric cancer risk in men (9). Women in the study and all other studies showed no association at all. When the anatomic subsite was considered, one study observed a moderate inverse association for distal cancer in women (11). On the other hand, case–

Table 1. Gastric cancer risk and consumption of green tea in cohort studies of Japanese populations

References	Study period	Study population				Category	No. among cases	Relative risk (95% CI or *P*)	*P* for trend	Confounding variables considered	Comments
Author		No. of subjects for analysis	Source of subjects	Event followed	No. of incident cases or deaths						
Nakachi et al. (8)	1986–99	8552	Population-based Saitama Prefecture	Death	140	Green tea, cups/day				Sex and lifestyle factors	
						≤3		1.0			
						≥10		0.69 (0.23–1.88)			
Tsubono et al. (9)	1984–92	26 311	Population-based Miyagi Prefecture	Incidence	419	Green tea, cups/day				Age, sex, types of health insurance, history of peptic ulcer, smoking status, alcohol consumption, daily consumption of rice, black tea, coffee, meat, green or yellow vegetables, pickled vegetables, other vegetables, fruits and bean-paste soup	
		11 902 men			296 men	Total					
		14 409 women			123 women	<1	66	1.0			
						1–2	68	1.1 (0.8–1.6)			
						3–4	79	1.0 (0.7–1.4)			
						≥5	206	1.2 (0.9–1.6)	0.13		
						Men					
						<1	41	1.0			
						1–2	49	1.3 (0.8–1.9)			
						3–4	55	1.2 (0.8–1.8)			
						≥5	151	1.5 (1.0–2.1)	0.03		
						Women					
						<1	25	1.0			
						1–2	19	0.8 (0.5–1.5)			
						3–4	24	0.7 (0.4–1.3)			
						≥5	55	0.8 (0.5–1.3)	0.46		
Hoshiyama et al. (10)	Mean 8 years	72 851	Population-based 45 areas of Japan	Death	359	Green tea, cups/day				Age, smoking status, history of peptic ulcer, family history of stomach cancer, consumption of rice, miso soup, green– yellow vegetables, white vegetables, fruits and preference for salty foods	
		30 370 men			240 men	Men					
		42 481 women			119 women	<1	24	1.0			
						1–2	51	1.6 (0.9–2.9)			
						3–4	51	1.1 (0.6–1.9)			
						5–9	76	1.1 (0.6–1.9)			
						≥10	38	1.0 (0.5–2.0)	0.634		
						Women					
						<1	20	1.0			
						1–2	18	1.1 (0.5–2.5)			
						3–4	40	1.0 (0.5–2.1)			
						5–9	32	0.8 (0.4–1.6)			
						≥10	9	0.7 (0.3–2.0)	0.476		

Continued

Table 1. *Continued*

References	Study period	Study population				Category	No. among cases	Relative risk (95% CI or *P*)	*P* for trend	Confounding variables considered	Comments
Author		No. of subjects for analysis	Source of subjects	Event followed	No. of incident cases or deaths						
Sasazuki et al. (11)	1990–2001	72,943	Population-based	Incidence	892	Green tea, cups/day				Age, area, cigarette smoking, consumption of fruits, green or yellow vegetables, fishgut, miso soup, black tea and coffee	
		34,832 men			665 men	Men					
		38,111 women			227 women	All sites					
						<1		1.0			
						1–2		0.95 (0.72–1.22)			
						3–4		0.84 (0.65–1.08)			
						≥5		0.98 (0.77–1.25)	0.65		
						Upper-third including cardia					
						<1		1.0			
						1–2		1.06 (0.51–2.18)			
						3–4		0.73 (0.34–1.57)			
						≥5		1.17 (0.60–2.30)	0.75		
						Distal					
						<1		1.0			
						1–2		0.88 (0.64–1.20)			
						3–4		0.79 (0.59–1.07)			
						≥5		0.92 (0.69–1.22)	0.37		
						Women					
						All sites					
						<1		1.0			
						1–2		0.85 (0.53–1.38)			
						3–4		1.04 (0.68–1.58)			
						≥5		0.67 (0.43–1.04)	0.08		
						Upper-third including cardia					
						<1		1.0			
						1–2					
						3–4		0.89 (0.34–2.33)			
						≥5			0.81		
						Distal					
						<1		1.0			
						1–2		0.88 (0.52–1.49)			
						3–4		1.00 (0.63–1.59)			
						≥5		0.51 (0.30–0.86)	0.01		

Khan et al. (12)	1984–2002	3158	Population-based Hokkaido	Death	51	Men			Age, smoking, health status, health education, health screening
		1524 men			36 men	Green tea ≤ several times/month		1.0	
		1634 women			15 women	Green tea ≥ several times/week		1.1 (0.4–2.5)	
						Women			
						Green tea ≤ several times/month		1.0	
						Green tea ≥ several times/week		0.7 (0.2–2.9)	
Sauvaget et al. (13)	1980–99	38 576	Atomic-bomb survivors: Hiroshima, Nagasaki	Incidence	1270	Green tea, cups/day			Sex, sex-specific age, city, radiation dose, sex-specific smoking habits and education level
		14 885 men				<2	242	1.0	
		23 691 women				2–4	680	1.03 (0.89–1.19)	
		34–98 years old				≥5	348	1.06 (0.89–1.25)	>0.50
Kuriyama et al. (14)	All-cause	40 530	Population-based	Death	193	Green tea, cups/day			Age, sex, job status, years of education, BMI, sports or exercise, walking duration, history of HT, DM, GU, smoking, alcohol, total energy, rice, miso soup, soy bean product, total meat, total fish, dairy products, total fruits, total vegetables, oolong tea, black tea, and coffee
	1995–2005 (11 years)	19 060 men				<1	44	1.0	
		21 470 women				1–2	44	1.33 (0.86–2.04)	
	Cause-specific					3–4	38	1.00 (0.64–1.58)	
	1995–2001 (7 years)					5≤	67	1.17 (0.78–1.76)	0.72
					138 men	<1	32	1.0	
						1–2	30	1.29 (0.78–2.16)	
						3–4	30	1.19 (0.71–2.00)	
						≥5	46	1.20 (0.74–1.95)	0.55
					55 women	<1	12	1.0	
						1–2	14	1.32 (0.59–2.94)	
						3–4	8	0.64 (0.26–1.63)	
						≥5	21	1.08 (0.50–2.33)	0.84
Suzuki et al. (15)	1999–2006	12 251	Population randomly chosen from all 74 municipalities in Shizuoka Prefecture	Death	68	Green tea, cups/day			Age, sex, smoking, alcohol, BMI, and physical activity
		6231 men				<1	2	1.0	Test for trend: HR = 1.04 (0.95–1.13)
		6020 women				1–3	14	0.49 (0.11–2.28)	
		65–84y old				4–6	32	0.78 (0.19–3.30)	
						≥7	20	0.81 (0.18–3.54)	

Continued

Table 1. *Continued*

References	Study period	Study population				Category	No. among cases	Relative risk (95% CI or *P*)	*P* for trend	Confounding variables considered	Comments
Author		No. of subjects for analysis	Source of subjects	Event followed	No. of incident cases or deaths						
Pooled analysis of 6 cohort studies including those listed above (9,10, cohort I of 11, cohort II of 11) or mentioned in the text (20 and 21)											
Inoue et al. (16)	1985–2004	219 080	Population-based	Incidence	3577	Green tea, cups/day					
		100 479 men			2495 men	Men				Age, area (for three cohorts only), smoking, alcohol drinking, rice, soy bean paste soup, coffee, pickled vegetables, and green-yellow vegetables intake	
		118 601 women			1082 women	All sites					
						<1	420	1.0			
						1–2	452	0.97 (0.83–1.12)			
						3–4	610	0.93 (0.81–1.08)			
						≥5	1013	1.06 (0.86–1.30)	0.74		
						Proximal (upper third)					
						<1	38	1.0			
						1–2	41	1.10 (0.70–1.73)			
						3–4	42	0.79 (0.46–1.35)			
						≥5	96	1.43 (0.96–2.14)	0.08		
						Distal (lower two-thirds)					
						<1	185	1.0			
						1–2	185	0.91 (0.73–1.12)			
						3–4	249	0.95 (0.77–1.16)			
						≥5	328	0.96 (0.79–1.17)	0.86		
						Women					
						All sites					
						<1	215	1.0			
						1–2	174	0.90 (0.73–1.10)			
						3–4	303	0.92 (0.76–1.11)			
						≥5	390	0.79 (0.65–0.96)	0.04		
						Proximal (upper third)					
						<1	8	1.0			
						≥1	45	1.17 (0.52–2.60)	0.87		
						Distal (lower two thirds)					
						<1	83	1.0			
						1–2	64	0.80 (0.57–1.13)			
						3–4	117	0.96 (0.71–1.30)			
						≥5	106	0.70 (0.50–0.96)	0.04		

NS, not significant; BMI, body mass index; HT, hypertension; DM, diabetes mellitus; GU, gastric ulcer.

Table 2. Gastric cancer risk and consumption of green tea in case–control studies of Japanese populations

References	Study time	Study subjects				Category	Relative risk (95% CI)	*P* for trend	Confounding variables considered	Comments
Author		Type and source	Definition	No. of cases	No. of controls					
Tajima and Tominaga (17)	1981–83	Hospital-based (Aichi Cancer Center)	Cases:	93	186	Green tea, times/day			Matched for age (±5 years), sex, time of interviews (±6 months)	
			Histologically confirmed cases			≥4	0.64	NS		
			Controls:			≤3	1.0			
			Patients without stomach cancer							
Kono et al. (18)	1979–82	Hospital-based (Karatsu Stomach Institute)	Cases:	139	Hospital controls: 2574	vs. hospital controls				
			Newly diagnosed as having gastric cancer at the Institute	74 men		Green tea, cups/day				
				65 women	1171 men	None or 1–4	1.0		Age, sex	
					1403 women	5–9	1.1			
						≥10	0.6	NS		
			Hospital controls:		General controls;					
			Patients without gastric cancer		278	≤9	1.0		Age, sex, smoking, oranges, fruits	
					148 men	≥10	0.5 (0.3–1.1)			
			General population controls:		130 women					
			Random sampling from the computerized file of residents			vs. general controls			General population:	
						Green tea, cups/day			Matched (1:2) for	
						None or 1–4	1.0		Sex	
						5–9	1.2		Age	
						≥10	0.4*	NS		
						≤9	1.0		Smoking, oranges, fruits	
						≥10	0.3 (0.1–0.7)†			

Continued

Table 2. *Continued*

References	Study time	Study subjects			Category	Relative risk (95% CI)	*P* for trend	Confounding variables considered	Comments
Author		Type and source	Definition	No. of cases	No. of controls				
Inoue et al. (19)	1990–95	Hospital-based (Aichi Cancer Center)	Cases: Histologically diagnosed cases of gastric cancer at the Institute	893	21 128	Green tea			
						Rarely	1.00		Coffee intake, black tea intake, gender, age, year and season at first hospital visit, habitual smoking, habitual alcohol drinking, regular physical exercise, fruit intake, rice intake, and beef intake
						Occasional	1.00 (0.77–1.44)		
						Daily			
			Controls:			1–3 cups/day	0.96 (0.70–1.32)		
			Outpatients without cancer			4–6 cups/day	1.01 (0.74–1.39)		
						≥7 cups/day	0.69 (0.48–1.00)‡		

NS, not significant.
*$P < 0.05$.
†$P = 0.007$.
‡$P < 0.05$.

control studies consistently showed a weak negative association between intake of green tea and risk of gastric cancer (Table 4). Among them, when using the general population as a control setting, Kono et al. (18) observed a strong negative association between green tea intake and gastric cancer risk.

During the review process, we were aware of the difference in effect by sex. In all studies that presented the analysis for men and women separately, although not statistically significant, the point estimate of highest category of green tea intake for women was consistently lower than that for men; i.e. based on incidence data, compared with <1 cup/day, the RRs of drinking ≥5 cups/day for men and women were estimated as 1.5 and 0.8 (9) and 0.98 and 0.67 (11), respectively (Table 1). Based on mortality data, the corresponding values for men and women were estimated as 1.0 and 0.7 (<1 vs. ≥10 cups/day) (10), and 1.1 and 0.7 (less than several times per month vs. more than several times per week) (12), respectively (Table 1). These results suggest a small protective effect, if any, of green tea intake and development of gastric cancer for women. However, applying our definition of magnitudes of association, they slightly failed to reach the level of weak association (weak association for 0.5 to <0.67, not significant). The null association observed among men may, in part, reflect insufficient adjustment for confounding factors such as cigarette smoking. Likewise, differences in the effect of green tea by subsite may point to an inconsistent effect on gastric cancer overall (11). However, evidence for such specific issues is sparse, probably due to the relatively small number of gastric cancer cases occurring in the upper subsite among cohorts, particularly in women. Results from pooled analysis may lead to a better understanding of these unresolved issues.

In a pooled analysis of six cohort studies (9–11,13,20,21) involving total of 219 080 subjects and 3577 gastric cancer cases, the role of green tea intake and gastric cancer risk was analyzed for men and women separately, with consideration of smoking status, anatomic subsite and so on (16). As a result, a statistically significant, weakly decreased risk of gastric cancer with ≥5 cups/day of green tea intake among women was observed [hazard ratio (HR) = 0.79, 95% confidence interval (CI) 0.65–0.96], although no association was observed among men (Tables 1 and 3). When the anatomic subsite was considered among four cohort studies in which the data were routinely collected, the risk reduction among women was more prominent in the distal gastric region (HR = 0.70, range 0.50–0.96; *P* for trend = 0.04). Together with the results of the systematic review, this finding from the pooled analysis was also considered to finally evaluate the evidence for green tea intake and gastric cancer risk in Japanese.

A difference in the effect of green tea intake by sex has also been observed for cardiovascular disease (14,22,23). The exact reason for the difference is unknown but may be explained, in part, by residual confounding effects of

smoking, phytoestrogens in tea and so on. It was suggested in some studies (9,14), but not all (16), that cigarette smoking might modify the effect of green tea. Tsubono et al. (9) observed a trend toward a positive association between green tea consumption and the risk of gastric cancer in subjects currently smoking ≥20 cigarettes/day (P for trend = 0.06), but not in other groups (P for the interaction term = 0.17). A similar interaction was suggested among studies investigating green tea intake and risk for cardiovascular disease (14). Higher rates of smoking may mask the effect of green tea consumption in men. Tea flavonoids such as kaempferol have been shown to exhibit estrogenic activity *in vitro* (24). In addition, tea contains lignan polyphenols, such as secoisolaracinol, which are considered phytoestrogenic (25). The phytoestrogens in tea might also partly account for the stronger protective effect of green tea in women than in men (26,27), although an estrogen-related protective mechanism against gastric cancer, if any, warrants further investigation.

Several aspects need to be discussed in relation to interpreting the present findings. Although cigarette smoking, which is suggested to have an interactive effect with green tea, was adjusted in most studies, *H. pylori* infection, a Group 1 carcinogen recognized by the International Agency for Research on Cancer (IARC), was not considered in any study. Based on the same origin of cohort study (10), Hoshiyama et al. (28) investigated whether green tea has any association with gastric cancer risk with considering *H. pylori* infection in a nested case–control study design within 157 incidence cases and 285 controls. They found that green tea intake had no protective effect against gastric cancer even after controlling for *H. pylori* infection. A previous nested case–control study that investigated plasma tea polyphenols and risk for gastric cancer reported that the decreased risk of gastric cancer by intake of tea polyphenols observed among women in the study remained even after adjusting for *H. pylori* infection (29). Some researchers used an animal model to report the inhibition of *H. pylori* urease by green tea extract (30) and the bactericidal effect on *H. pylori* infection by green tea catechins (31). A long-term habit of drinking green tea might lead to the elimination of *H. pylori*; if this is true, *H. pylori* may act as an intermediate rather than a confounding factor in the relationship between green tea and gastric cancer.

Discrepancies were noted in the effects of green tea on gastric cancer risk between case–control studies and cohort studies. The discrepancies were quantitatively shown from a recent meta-analysis of green tea intake and gastric cancer risk based on 13 studies from Japan and China (32). Compared with the lowest level of green tea intake, the RR of gastric cancer for the highest level of green tea intake was 0.73 (95% CI 0.64–0.83) for case–control studies, whereas no association was observed for cohort studies (RR = 1.04, 95% CI 0.93–1.17). The discrepancy may be partially explained by recall or selection biases that are inevitable in case–control studies. For example, it is possible that individuals with gastric cancer reduce their green tea intake due to their gastric symptoms. In fact, it has been reported that among those with gastric cancer, black tea consumption was reduced even up to 2 years before their diagnosis was made (33). Therefore, the green tea intake for gastric cancer cases among case–control studies might be partly underreported. Another point is the variation of gastric cancer mortality rates across the country. The age-adjusted gastric cancer mortality rate under age 75 in 2009 in Japan was 11.8/100 000 and ranged from 6.3 (Okinawa prefecture) to 15.7 (Akita prefecture) (34). It is interesting that gastric cancer mortality rates in the two prefectures in the case–control studies are higher than the average level, whereas the situation for cohort studies is mixed. On the basis of wide variation in gastric cancer mortality rates by area, the approach such as pooled analysis might be important.

However, the null results in cohort studies also contradict the results of previous experimental studies that suggested the protective effect of tea polyphenols on gastric cancer using *in vivo* animal models and *in vitro* cancer cell lines (3). In most of the cohort studies where the validity of green tea intake was examined, a moderate validity was shown; the Spearman coefficient for the correlation between the green tea intake according to the questionnaire and the amounts consumed according to the food records ranged from 0.29 to 0.71 (9–11,13,14). However, in all epidemiologic studies investigating green tea intake and gastric cancer risk, green tea consumption was determined only in terms of self-reported frequency of drinking, and the size of the cup was not ascertained. Furthermore, the amount of tea polyphenols in one cup varies according to preparation, i.e. the type and amount of green tea leaves, the frequency of renewing a tea batch in the pot, the temperature of boiled water or time to brew the tea and so on. A number of studies have found that hot drinks have an effect on the risk for esophageal cancer (35). Yu et al. (36) also showed that boiling hot tea had a non-significant increased risk of causing gastric cancer (odds ratio = 1.18). The risk estimates for the cardia, pylori and antrum sites regarding boiling hot tea were 2.09, 0.56 and 0.82, respectively. Furthermore, the term 'green tea' might be ambiguous because some participants may include only 'sencha', which looks green, or others may also include 'bancha/houjicha/genmaicha', which is also a commonly consumed Japanese tea but looks brown. Sencha, one of the most popular green teas in Japan, contains higher levels of tannin, vitamin C and folate than bancha/houjicha/genmaicha (37). Inaccurate measurement of green tea consumption in epidemiologic studies necessarily attenuates the small effect of green tea. It is interesting that both studies using biomarkers of green tea intake showed a statistically significant association with gastric cancer. Sun et al. (38) reported that urinary (−)-epigallocatechin (EGC) showed a statistically significant inverse association with gastric cancer. In a case–control study nested within a cohort study (11), a high plasma level of EGC was associated with an increased risk of gastric cancer in men, whereas a high plasma level of

Table 3. Summary of associations between gastric cancer risk and consumption of green tea in cohort studies of Japanese populations

References			Study period	Study subjects					Strength of association
Author	Year	Ref. no.		Sex	No. of subjects	Age range	Event	No. of cases	
Nakachi et al.	2000	8	1986–99	Men and women	8552	40+	Death	140	–
Tsubono et al.	2001	9	1984–92	Men	11 902	40+	Incidence	296	↑
				Women	14 409			123	–
Hoshiyama et al.	2002	10	1988–97	Men	30 370	40–79	Death	240	–
				Women	42 481			119	–
Sasazuki et al.	2004	11	1990–2001	Men	34 832	40–59	Incidence	665	–
				Women	38 111			227	– (distal ↓↓)
Khan et al.	2004	12	1984–2002	Men	1524	40+	Death	36	–
				Women	1634			15	–
Sauvaget et al.	2005	13	1980–99	Men and women	38 576	34–98	Incidence	1270	–
Kuriyama et al.	2006	14	1995–2001	Men	19 060	40–79	Death	138	–
				Women	21 470			55	–
Suzuki et al.	2009	15	1999–2006	Men and women	12 251	65–84	Death	68	–
Pooled analysis of 6 cohort studies including those listed above (9,10, cohort I of 11, cohort II of 11) or mentioned in the text (20 and 21)									
Inoue et al.	2009	16	1985–2004	Men	100 479	40–103	Incidence	2495	–
				Women	118 601			1082	↓

Explanation for each symbol is as follows when statistical significance (SS) or no statistical significance (NS), strong (symbol ↓↓↓ or ↑↑↑), <0.5 or >2.0 (SS); moderate (symbol ↓↓ or ↑↑), either (i) <0.5 or >2.0 (NS), (ii) >1.5–2 (SS) or (iii) 0.5 to <0.67 (SS); weak (symbol ↓ or ↑), either (i) >1.5–2 (NS), (ii) 0.5 to <0.67 (NS) or (iii) 0.67–1.5 (SS); or no association (symbol –), 0.67–1.5 (NS).

Table 4. Summary of associations between gastric cancer risk and consumption of green tea in case–control studies of Japanese populations

References			Study period	Study subjects				Strength of association
Author	Year	Ref. no.		Sex	Age range	No. of cases	No. of controls	
Tajima and Tominaga	1985	17	1981–83	Men and women	40–70	93	186	↓
Kono et al.	1988	18	1979–82	Men and women	20–75	139	Hospital 2547	↓
							General population 278	↓↓↓
Inoue et al.	1998	19	1990–95	Men and women	40+	893	21 128	↓

Explanation for each symbol is as follows when statistical significance (SS) or no statistical significance (NS), strong (symbol ↓↓↓ or ↑↑↑), <0.5 or >2.0 (SS); moderate (symbol ↓↓ or ↑↑), either (i) <0.5 or >2.0 (NS), (ii) >1.5–2 (SS) or (iii) 0.5 to <0.67 (SS); weak (symbol ↓ or ↑), either (i) >1.5–2 (NS), (ii) 0.5 to <0.67 (NS) or (iii) 0.67–1.5 (SS); or no association (symbol –), 0.67–1.5 (NS).

(−)-epicatechin-3-gallate was associated with a statistically significant decreased risk of gastric cancer in women (29).

In conclusion, we found no preventive effect on gastric cancer for green tea intake in cohort studies, which have fewer biases and are more persuasive than case–control studies, where risk reduction was shown. However, a small, consistent risk reduction limited to women was observed, which was confirmed by pooling data from six cohort studies.

EVALUATION OF EVIDENCE ON GREEN TEA CONSUMPTION AND GASTRIC CANCER RISK IN JAPANESE

From the results of the systematic review and pooled analysis of green tea intake and gastric cancer risk and on the basis of assumed biologic plausibility, we conclude that green tea possibly decreases the risk of gastric cancer in women. However, epidemiologic evidence is still insufficient to demonstrate any association in men.

Acknowledgements

The authors gratefully acknowledge the assistance of Ms Izumi Suenaga in this report.

Funding

This study was supported by the Third Term Comprehensive 10-year Strategy for Cancer Control from the Ministry of Health, Labour and Welfare, Japan.

Conflict of interest statement

None declared.

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Appendix

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Isoflavone intake and risk of gastric cancer: a population-based prospective cohort study in Japan[1–3]

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ABSTRACT

Background: Isoflavones are structurally similar to 17β-estradiol and may be able to prevent gastric cancer. However, there is contradictory evidence concerning the relation between the intake of soy food, which is rich in isoflavones, and gastric cancer. The association with gastric cancer might differ between isoflavones and soy foods, and research on the effects of isoflavone intake alone on gastric cancer is needed.

Objective: We investigated the association between isoflavone intake and the incidence of gastric cancer.

Design: We conducted a large, population-based prospective study of 39,569 men and 45,312 women aged 45–74 y. Dietary soy and isoflavone intakes were measured by using a validated food-frequency questionnaire in 1995 and 1998.

Results: During 806,550 person-years of follow-up, we identified 1249 new gastric cancer cases. Isoflavone intake was not associated with gastric cancer in either men or women. Compared with the lowest quartile, the HR and 95% CI for developing gastric cancer in the fourth quartile of isoflavone intake was 1.00 (0.81, 1.24) for men and 1.07 (0.77, 1.50) for women. In a stratified analysis by exogenous female hormones (women only), however, we found an increasing trend in risk of gastric cancer associated with higher isoflavone intakes among exogenous female hormone users (P-trend = 0.03) but not for nonusers (P-interaction = 0.04).

Conclusion: The current study does not support the hypothesis that higher intakes of isoflavones prevent gastric cancer in either men or women. *Am J Clin Nutr* 2012;95:147–54.

INTRODUCTION

Although its incidence and mortality rate have been declining over the years (1), GC[4] is still the most common cancer in Japan and the second leading cause of death from cancer globally. Prevention of GC is one of the most important elements for cancer control strategy both in Japan and around the world.

Sex-based discrepancies in GC are found throughout the world, and the incidence of GC in men is 2- to 3-fold that in women (2). This difference is consistent across international populations regardless of different prevalences of environmental risk factors, such as *Helicobacter pylori* infection, tobacco smoking, and different dietary patterns (1, 3). A possible explanation involves biologic differences related to sex hormones, such as estrogen (3).

Isoflavones are structurally similar to 17β-estradiol, have a particular affinity for the β-estrogen receptor (4), and may be able to prevent GC. Because isoflavones are phytoestrogenic compounds that are abundant in soybeans, soy products have been of considerable interest in the etiology of GC (5). However, evidence of the relation between soy food intake and GC is contradictory. Non-isoflavone aspects of soy food, such as salt intake and fermentation, might contribute to the different association with GC between soy food and isoflavones, because salt is a well-known risk factor for GC (6), and fermented soy foods may contain nitroso compounds, which have been reported to induce gastric carcinogenesis (7, 8). Therefore, the association of isoflavones with GC might be different from that of soy food, and further research on the effects of isoflavones alone on GC is needed. However, no large-scale prospective study to assess this association has been conducted.

Here, we investigated the association between isoflavone intake and risk of GC in a population-based, prospective, cohort study in Japan. Our hypothesis was that a higher intake of isoflavones would prevent GC because of their estrogen-like effects.

SUBJECTS AND METHODS

Study population

The JPHC-Based Prospective Study was started in 1990 for cohort I and in 1993 for cohort II. Subjects were all registered Japanese inhabitants in 11 public health center areas who were aged 40–69 y (cohort 1: 40–59 y; cohort 2: 40–69 y) at the beginning of each cohort's baseline survey. Details of the study

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[2] Supported by Management Expenses Grants from the Government to the National Cancer Center, Grants-in-Aid for Cancer Research, and the Third Term Comprehensive 10-Year Strategy for Cancer Control (H21-Sanjigan-Ippan-003) from the Ministry of Health, Labor, and Welfare of Japan. AH received a research resident fellowship from the Foundation for Promotion of Cancer Research (Japan) for the third term Comprehensive 10-year Strategy for Cancer Control.

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[4] Abbreviations used: EFH, exogenous female hormones; FFQ, food-frequency questionnaire; GC, gastric cancer; JPHC, Japan Public Health Center.

Received June 1, 2011. Accepted for publication October 12, 2011.

First published online December 14, 2011; doi: 10.3945/ajcn.111.020479.

design were described previously (9). The institutional review board of the National Cancer Center, Tokyo, Japan, approved the study. The participants in the current study were subjects in the JPHC study who responded to a 5-y follow-up questionnaire in 1995–1999 at the age of 45–74 y. This follow-up survey was used as the starting point in the current study. The subjects from 2 public health center areas (Katsushika in Tokyo prefecture and Suita in Osaka prefecture) were excluded from the current analysis because the selection of subjects was different from that in other public health center areas, which left 116,896 subjects as the study population. After the exclusion of subjects with a non-Japanese nationality (n = 51), a late report of emigration occurring before the starting point (n = 168), or ineligibility due to incorrect birth date (n = 4) or duplicate enrollment (n = 4), we established a population-based cohort of 116,669 subjects. After the exclusion of 1625 subjects who had died, moved out of the study area, or were lost to follow-up before the starting point, 115,044 eligible subjects remained. Of these, 91,246 responded to the questionnaire, which yielded a response rate of 78.2%.

Questionnaire

We asked the subjects to reply to a lifestyle questionnaire that covered sociodemographic characteristics, medical history, smoking and drinking habits, diet, and other characteristics. We designed the FFQ to estimate dietary intake from 138 food items and validated it for the estimation of various nutrients and food groups (10). The participants were asked about how often they consumed the individual food items (frequency of intake) and to estimate representative relative sizes compared with standard portions during the previous year (11). Of the 138 food items, 8 items (standard portion size) dealt specifically with consumption of soy and isoflavones: miso soup (150 g), soymilk (200 g), tofu for miso soup (20 g), tofu for other dishes (75 g), *yushidofu* (predrained tofu; 150 g), *koyadofu* (freeze-dried tofu; 60 g), *aburaage* (deep-fried tofu; 2 g), and *natto* (fermented soybeans; 50 g). These 8 items contributed 95.9% of the total genistein and daidzein intakes in the estimates from dietary records in our validation study (12). We defined fermented soy food as miso (for miso soup) and *natto*, whereas nonfermented soy food was defined as soymilk, tofu for miso soup, tofu for other dishes, *yushidofu*, *koyadofu*, *and aburaage* (13). We then estimated genistein and daidzein intakes from either fermented or nonfermented foods. For miso soup, the FFQ included questions on the frequency of consumption (almost never, 1–3 d/mo, 1–2 d/wk, 3–4 d/wk, 5–6 d/wk, or daily) and on the daily amount consumed (number of bowls: <1, 1, 2, 3, 4, 5, 6, 7–9, or ≥ 10). For soymilk, the FFQ included questions on 10 frequency categories only: almost never, 1–3 times/mo, 1–2 times/wk, 3–4 times/wk, 5–6 times/wk, 1 glass/d, 2–3 glasses/d, 4–6 glasses/d, 7–9 glasses/d, or ≥ 9 glasses/d. For other soy foods, the FFQ contained questions on frequency (almost never, 1–3 times/mo, 1–2 times/wk, 3–4 times/wk, 5–6 times/wk, 1 time/d, 2–3 times/d, 4–6 times/d, or ≥ 7 times/d) and sizes relative to a standard portion [small (50% smaller than standard), medium (same as standard), or large (50% larger than standard)].

The daily intake of each food item was calculated by multiplying the frequency by the standard portion and, if available, the relative portion size for each item in the FFQ. We calculated daily intakes of isoflavones (genistein and daidzein) using values in a specially developed food-composition table of Japanese foods (14), which contained measured values of soy foods (15, 16). This allowed for the effect of food processing on isoflavone content, including fermentation, to be taken into consideration when intakes were estimated. We did not collect information on the use of isoflavone supplements. Intake of food and nutrients was log transformed and adjusted for total energy intake by using the residual model (17). Because the estimates of genistein and daidzein intakes were highly correlated (Spearman's rank correlation coefficient = 0.997), the results for genistein are provided as representative for isoflavones.

The validity of the energy-adjusted genistein intake assessed from the 5-y FFQ was evaluated in a subsample with consecutive 14- or 28-d dietary records. Spearman's correlation coefficients between the energy-adjusted intake of genistein from the questionnaire and from dietary records was 0.65 (cohort I) and 0.48 (cohort II) for men and 0.55 (cohort I) and 0.45 (cohort II) for women (18–21). The reproducibility between the 2 questionnaires for energy-adjusted genistein intake assessed 1 y apart showed Spearman's correlation coefficients of 0.75 (men) and 0.69 (women) for cohort I and 0.51 (men) and 0.41 (women) for cohort II (18–21).

We excluded subjects with a diagnosis of GC or who reported having GC before the starting point (n = 746), who had missing data regarding isoflavone intake (n = 1115), or who reported extreme total energy intakes (upper: 2.5%; lower: 2.5%) (n = 4504). The final analysis included 84,881 subjects (39,569 men and 45,312 women).

Follow-up and identification of GC cases

We followed subjects from the 5-y follow-up survey until 31 December 2006. We identified changes in residence status, including survival, annually through the residential registry in each area or, for those who had moved out of the area, through the municipal office of 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 database. 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 current study, 9370 (11.0%) subjects died, 3675 (4.3%) moved out of the study area, and 305 (0.4%) were lost to follow-up.

We identified incident data for GC by active patient notification from major local hospitals in the study area and from data linkage with population-based cancer registries. We coded GC cases according to the International Classification of Diseases for Oncology, third edition (22) (C16.0–C16.9). Tumors located in the lower side of the stomach were classified as distal GC (noncardia; code C16.2–16.7) and in the upper side as proximal GC (cardia; code C16.0–16.1). Tumors that could not be classified because they were overlapping lesions (code C16.8) or because no information was available (code C16.9) were categorized as unclassified. Histologic classification was based on review of the record from the respective hospital as described previously (23) and divided into differentiated and undifferentiated types, corresponding to the intestinal type and diffuse type, respectively, in the Lauren classification (24). In our