# Plasma Tea Polyphenols and Gastric Cancer Risk: A Case-Control Study Nested in a Large Population-Based Prospective Study in Japan

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## Abstract

**Abundant *in vitro* and animal studies have shown a protective effect of green tea against various types of cancer, but the evidence from epidemiologic studies is inconclusive. In this nested case-control study, we used plasma biomarkers to directly investigate the effect of tea polyphenols on the risk of gastric cancer. Subjects were followed up from 1990 to 2004. Among 36,745 subjects who answered the baseline questionnaire and provided blood samples, 494 gastric cancer cases matched to 494 controls were used in the analysis. The validated method used high-performance liquid chromatography to analyze baseline plasma samples. For men, a high plasma level of (-)-epigallocatechin was associated with an increased risk of gastric cancer. For women, a high plasma level of (-)-epicatechin-3-gallate (ECG) was associated with a decreased risk of gastric cancer; the adjusted odds ratios (95% confidence intervals) for ECG levels 0.32 to 9.2 and 9.3+ ng/mL were 1.03 (0.41-2.59) and 0.25 (0.08-0.73), respectively, compared with those whose ECG level was under the detection limit (*P* for trend = 0.02). Cigarette smoking was suggested to play a role as an effect modifier, which explains in part the different patterns observed by gender.** (Cancer Epidemiol Biomarkers Prev 2008;17(2):343–51)

## Introduction

Tea is one of the most popular beverages around the world. Green tea is primarily consumed in Asian countries, especially in Japan and China, and in some parts of North Africa and the Middle East (1). Green tea contains polyphenols, which are commonly known as catechins. Some major green tea catechins are (-)-epigallocatechin-3-gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG), and (-)-epicatechin (EC). Antioxidant activities and the ability to inhibit nitrosation of polyphenols have been isolated from green tea in *in vitro* and *in vivo* studies (2-4). In addition, recent research has proposed many other possible mechanisms for the cancer-inhibiting effects of green tea. These mechanisms include modulation of signal transduction pathways (which leads to inhibition of cell proliferation and transformation), induction of apoptosis and cell cycle arrest, and inhibition of tumor invasion and angiogenesis (5-7). Although abundant *in vitro* and animal studies have shown that green tea has a protective effect against some types of cancer, the evidence from epidemiologic studies is inconclusive (8). One reason for inconclusive results may be the crude assessments of green tea, especially polyphenols, in epidemiologic studies. In most studies, green tea consumption was determined in terms of self-reported frequency of drinking only, and the size of cup was not ascertained. Furthermore, the amount of tea polyphenols in one cup varies according to preparation, the type and amount of green tea leaves, the frequency of renewing a tea batch in the pot, temperature of the boiled water or time to brew the tea, etc. Inaccurate measurement of green tea consumption necessarily attenuates the true association with green tea. Interestingly, among six studies conducted in China in which green tea was assessed through the amount of green tea leaves consumed, five reported a decreased risk of gastric (9-11), colorectal (12), esophageal (13), and pancreatic (12) cancers associated with green tea.

Because EGCG, EGC, ECG, and EC are not present in substantial amounts in foods or beverages besides tea, measuring these catechins in biological fluids or tissues may be one way to assess green tea intake directly. So far, only one study has used biomarkers to investigate the relationship between green tea and gastric cancer. Sun et al. (14) reported that urinary EGC showed a statistically significant inverse association with gastric cancer after exclusion of cases diagnosed sooner than 4 years after follow-up. However, EGCG, the main catechin, is not detectable in urine. There is now a validated assay for quantifying plasma EGCG, EGC, ECG, and EC (15, 16). In this nested case-control study, we used plasma biomarkers to directly investigate the effect of tea polyphenols on the risk of gastric cancer. This is the first study that has used plasma levels of tea polyphenols to show the relationship between green tea and gastric cancer risk.

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## Participants and Methods

**Study Population.** The Japan Public Health Center–based prospective study (JPHC Study) is an ongoing cohort study that investigates 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 (17). Study subjects were defined as all inhabitants in the study areas (27 cities, towns, or villages in nine public health centers) ranging in age from 40 to 59 years in cohort I and 40 to 69 years in cohort II. Moreover, a subcohort of health checkup examinees were included in cohorts I and II. Among these subjects, those registered at one public health center area in cohort I and one subcohort in cohort II were deleted from the present analysis, because data on cancer incidence were not available, and the method of selecting subjects differed from that of other cohort subjects, respectively. As a whole, a population-based cohort of 61,009 men (27,062 in cohort I and 33,947 in cohort II) and 62,567 women (27,436 in cohort I and 35,131 in cohort II) was established.

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 to 1994 for cohort II, subjects were asked to reply to a lifestyle questionnaire that covered sociodemographic characteristics, medical history, smoking and drinking habits, and diet. Details of the food frequency questionnaire included in the baseline survey have been described previously (18). Briefly, dietary questions measured the usual consumption of 44 food items during the previous year according to four categories (rarely, 1-2 days/wk, 3-4 days/wk, and almost daily) for cohort I and 52 items according to five categories (none, rarely, 1-2 days/wk, 3-4 days/wk, and almost daily) for cohort II without standard portions. Daily nutrient intakes were calculated by multiplying the consumption frequency of each food by the nutrient content of the standard portions/units and summing these values for all foods. Total salt intake (g/d) was calculated by multiplying 58.5/23 by sodium. Validity was assessed among subsamples with the use of 14- or 28-day dietary records. Spearman correlation coefficients between two indices for sodium intake were 0.49 in men and 0.54 in women for cohort I (19) and 0.46 in men and 0.33 in women for cohort II.[1] With regard to the reproducibility of estimations between two questionnaires administered 1 year apart, respective correlation coefficients for sodium were 0.53 in men and 0.65 in women for cohort I (19). Body mass index (BMI) was derived from self-reported current height and weight values reported in the questionnaire. A total of 99,808 (81%) subjects (47,525 men and 52,283 women) responded to the questionnaires.

A total of 10 mL blood was provided voluntarily by each subject during the health checkups. The plasma and buffy layer were divided into four tubes, each holding 1.0 mL (three tubes for plasma and one for the buffy layer), and stored at −80°C. The blood was collected from 1990 to 1992 in cohort I and from 1993 to 1995 in cohort II.

We excluded subjects with a self-reported cancer at baseline ($n$ = 2,136), those who were not Japanese ($n$ = 18), and those who had already moved away at baseline ($n$ = 11). These exclusions left 46,803 eligible men and 50,841 women. Among them, 13,467 (29%) men and 23,278 (46%) women donated their blood samples at baseline and were included in the study.

### Follow-up and Identification of Gastric Cancer

*Death and Relocation.* Subjects were followed until December 31, 2004. The changes in residency status, including death, were identified annually through the residential registry in each area. To confirm the causes of death, we used mortality data from the Ministry of Health, Labour and Welfare. Among study subjects, 6,133 (6.3%) had relocated, 6,035 (6.2%) had died, and 82 (0.08%) were lost to follow-up within the study period.

*Cancer Registry for JPHC Study.* Newly diagnosed cases of cancer were collected from two data sources, one from local major hospitals and the other from population-based registries (usually prefecture-wide). Candidate patients were linked by name, address, and date of birth and entered in the cancer registry for the JPHC Study when the date of birth and residence fulfilled cohort inclusion criteria. In our cancer registry system, the proportion of cases for which information was available from death certificates only was 3.2% for gastric cancer.

*Identification of Gastric Cancer and Selection of Control Subjects.* Cases of gastric cancer were extracted from the cancer registry for the JPHC Study based on site (*International Classification of Diseases for Oncology* code C160-169; ref. 20). Up to the end of the study period, 512 new gastric cancer cases were identified.

For each case, we selected 1 control matched for gender, age (±3 years), study area, blood donation date (±2 months), and fasting time at blood donation (±5 h). The final analysis included 494 sets, with each set containing 1 case and 1 control (total of 494 cases and 494 controls), excluding 1 case and 17 cases with a technical error in the *Helicobacter pylori* antibody measurement and tea polyphenol measurement, respectively, and their matched controls.

### Laboratory Analysis

*Chemicals and Reagents.* EGC, EC, EGCG, ECG, β-glucuronidase (G-7896), and sulfatase (S-9754) were obtained from Sigma. Acetonitrile, methanol, disodium ethylenediamine triacetic acid, sodium acetate trihydrate, phosphoric acid, and distilled water were purchased from Wako Pure Chemical Industries. Sodium dihydrogen phosphate monohydrate was obtained from Merck. All solvents and chemicals were high-performance liquid chromatography grade or analytical grade.

*Analysis of Plasma Tea Polyphenols.* The plasma samples were analyzed by the modified method of Lee et al. (15, 16).

Plasma (400 μL) was buffered with 1 mL of 0.1 mol/L sodium phosphate buffer (pH 5.8). β-Glucuronidase (10 μL; 250 IU) and sulfatase (10 μL; 5 IU) solution were added to the mixture and then incubated at 37°C for 20 min to hydrolyze the conjugated form of the catechins. The samples were applied to the solid-phase extraction cartridge (Abselut-Nexus C18, 60 mg, 3 mL; Varian), then

[1] Unpublished data.

with 1.2 mL of 50 mmol/L sodium phosphate buffer (pH 3.0), followed by 1.2 mL of 50 mmol/L sodium phosphate buffer (pH 3.0) containing 5% methanol. The catechins were eluted with 1.2 mL methanol/acetonitrile (3:7), and this fraction was evaporated by a vacuum centrifugation. The residue was dissolved in 200 μL of 50% methanol, filtered (0.45 μm Ultrafree-MC low-binding Durapore centrifugal filter; Millipore), and then applied to the high-performance liquid chromatography system described below.

The high-performance liquid chromatography system consisted of ESA model 542 refrigerated autoinjector, ESA model 580 solvent delivery module, and ESA 5600A 8-channel coulometric electrode array detector (ESA). An octadecyl column (ODS250, I.D. 250 × 4.6 mm, 5-μm particle size; MC Medical) was used at 37°C. The mobile phase was mixed with two solvents: solvent A [0.1 mol/L phosphoric acid (pH 3.35)] and solvent B [0.1 mol/L phosphoric acid (pH 3.35)/methanol/acetonitrile (30:10:60, v/v/v)]. Separation was achieved by using a linear gradient elution of B into A at a flow rate of 1.0 mL/min (37°C; 0-10 min, 0% A to 8% B; 10-35 min, 8% A to 20% B; 35-70 min, 20% A to 60% B; 70-75 min, 60% A to 100% B; 75-90 min, 100% B). The setting potentials were 20, 60, 80, 140, 320, 400, 440, and 480 mV. Profiles of reactions in these 8 channels were used to identify the peaks of EGC, EC, EGCG, and ECG. The regression coefficient of peak height and concentration calculated for tea polyphenols showed linearities of 0 to 1.0 μg/mL, respectively, with correlation coefficient values greater than 0.996. The within-day variation of voltammetric response for the standard solution had a coefficient of variation range of 6.6% to 12.6%. The recovery rate of the tea polyphenols in the plasma samples ranged from 64.1% to 88.6% (EGC, 87.1%; EC, 88.6%; EGCG, 66.2%; and ECG, 64.2%). The limits of detection for tea polyphenols were (EGC, 1.37 ng/mL; EC, 1.58 ng/mL; EGCG, 1.77 ng/mL; and ECG, 0.32 ng/mL); data below these limits were regarded as zero.

IgG antibodies to *H. pylori* were measured with a direct ELISA kit (E Plate ''Eiken'' *H. pylori* Antibody; Eiken Kagaku). Levels of IgG were categorized as seropositive and seronegative for *H. pylori* according to a selective cutoff value (492 nm). All assays were conducted by a person blinded to the case-control situation.

**Statistical Analysis.** $\chi^2$ test or one-way ANOVA was used to see the distribution of several factors according to plasma levels of tea polyphenols in the controls (Tables 1 and 2). Matched odds ratios (OR) and 95% confidence intervals (95% CI) were calculated to indicate the relationship between each tea polyphenol level and the risk of gastric cancer. Multiple conditional logistic regression analyses were conducted to control for potential confounding factors, such as smoking status, consumption of salted fish preserves, salt, green and yellow vegetables, other vegetables, fruit, BMI, family history of gastric cancer, and *H. pylori* infection. In light of the importance of cigarette smoking as a confounding factor, an index that included both smoking intensity and duration (never smoker; past smoker with 10+ and 1-9 years of smoking cessation; and current smoker with 0.5-29.5, 30-39, and 40+ pack-years) was used. Pack-years were calculated by multiplying the years of smoking and the average numbers of cigarettes smoked per day then dividing this value by 20. Tea polyphenol plasma levels (T1-T3), the frequencies of consumption of each food, and BMI were categorized into three groups so that each category included an approximately equal number of male and female 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. Because adjustment for confounding factors did not significantly alter the results, only adjusted ORs are listed in the tables. The trend was assessed by assigning ordinal values for categorical variables. Interaction between cigarette smoking and tea polyphenols was tested by adding cross-product terms to the multivariate model. Reported *P* values were two sided, and all statistical analyses were done with SAS software version 9.1 (SAS Institute).

## Results

The baseline characteristics of the control subjects according to plasma levels of tea polyphenols are shown separately for men (Table 1) and women (Table 2). The percentage of men in whom polyphenols were not detected ranged from 50.5% (EGC) to 76.4% (ECG). For women, the percentage ranged from 49.1% (EGC) to 73.6% (ECG). In men, the Spearman rank correlation coefficients between green tea intake (as assessed by food frequency questionnaire) and plasma EGC, EC, EGCG, ECG levels were calculated as 0.33, 0.29, 0.31, and 0.24, respectively. The corresponding values for women were 0.24, 0.22, 0.22, and 0.17, respectively. In men, cigarette smoking was more frequent among those with higher levels of tea polyphenols. On the other hand, alcohol drinking showed no material variation in relation to the level of tea polyphenols. Age, dietary factors, family history of gastric cancer, *H. pylori* infection, and BMI did not distribute differentially in relation to tea polyphenol level. Consumption of other vegetables was more frequent among women whose tea polyphenol level was under the detection limit. *H. pylori* infection was more frequent among those with a low tea polyphenol level. Other factors showed no material variation in relation to tea polyphenol level.

Table 3 shows the matched ORs and 95% CIs of developing gastric cancer in relation to plasma levels of tea polyphenols separately in men and women.

For men, a high plasma level of EGC was associated with an increased risk of gastric cancer. After being adjusted for confounding factors, the matched ORs and 95% CIs for those with EGC levels T2 and T3 were 1.85 (1.15-2.97) and 2.06 (1.23-3.45), respectively, compared with those whose EGC level was under the detection limit (*P* for trend = 0.003). Increased risk was also suggested for the three other tea polyphenols. Cigarette smoking was also associated with an increased risk of gastric cancer with a statistically significant trend. The ORs for past smokers with 10+ and 1-9 years of smoking cessation and current smokers with 0.5-29.5, 30-39, and 40+ pack-years were 0.97, 1.53, 1.44, 1.79, and 1.82, respectively (*P* for trend = 0.01).

For women, a high plasma level of ECG was associated with a decreased risk of gastric cancer; the adjusted ORs and 95% CIs for women with ECG levels T2 and T3 were 1.03 (0.41-2.59) and 0.25 (0.08-0.73),

**Table 1. Baseline characteristics of male controls according to serum tea polyphenol level**

| | Plasma EGC level (ng/mL) | | | | Plasma EC level (ng/mL) | | | |
|---|---|---|---|---|---|---|---|---|
| | T1: 0 ($n$ = 167) | T2: 1.37-77.9 ($n$ = 81) | T3: 78.0+ ($n$ = 83) | $P$ for difference* | T1: 0 ($n$ = 200) | T2: 1.58-33.1 ($n$ = 65) | T3: 33.2+ ($n$ = 66) | $P$ for difference* |
| Age | 56.4 (1.3) | 57.6 (1.0) | 60.3 (1.3) | 0.24 | 58.2 (1.2) | 57.7 (1.1) | 58.3 (1.5) | 0.89 |
| Current smoking (%) | 39.5 | 44.4 | 50.6 | 0.25 | 39.5 | 49.2 | 50.0 | 0.19 |
| Alcohol consumption, 1+/wk (%) | 71.3 | 69.1 | 65.1 | 0.61 | 72.0 | 72.3 | 57.6 | 0.07 |
| Cod roe or fish gut, 1+ d/wk (%) | 41.3 | 35.8 | 42.2 | 0.65 | 39.5 | 35.4 | 47.0 | 0.38 |
| Total salt (g/d) | 5.66 (0.18) | 5.48 (0.26) | 5.06 (0.26) | 0.16 | 5.56 (0.17) | 5.71 (0.29) | 4.94 (0.29) | 0.12 |
| Green-yellow vegetables, daily (%) | 41.3 | 48.2 | 34.9 | 0.23 | 41.5 | 46.2 | 36.4 | 0.52 |
| Other vegetables, 3+/wk (%) | 47.9 | 44.4 | 33.7 | 0.1 | 46.0 | 47.7 | 31.8 | 0.1 |
| Fruit, 3+/wk (%) | 50.3 | 63.0 | 55.4 | 0.17 | 54.0 | 56.9 | 54.6 | 0.92 |
| Green tea, 3+ cups/d (%) | 44.3 | 70.4 | 72.3 | <0.0001 | 49.0 | 66.2 | 75.8 | 0.0002 |
| Family history of gastric cancer (%) | 9.0 | 8.6 | 6.0 | 0.71 | 8.5 | 7.7 | 7.6 | 0.96 |
| *H. pylori* infection (%) | 76.1 | 77.8 | 74.7 | 0.9 | 75.0 | 76.9 | 78.8 | 0.81 |
| BMI | 23.4 (0.5) | 23.0 (0.4) | 22.9 (0.5) | 0.75 | 23.2 (0.5) | 22.6 (0.4) | 23.4 (0.6) | 0.37 |

NOTE: Values are mean (SE), except as otherwise specified.
*Based on $\chi^2$ test or ANOVA.

respectively, compared with those whose ECG levels were under the detection limit ($P$ for trend = 0.02). The three other tea polyphenols were also thought to be related to a decreased risk of gastric cancer.

When the data for men and women were combined and divided by baseline smoking status, the relationship between levels of tea polyphenols and gastric cancer showed a different pattern (Table 4). For never or past smokers, most of the point estimates for the T3 level appeared to be under unity, especially with statistical significance for ECG in never smoker; the ORs for the T3 plasma levels of EGC, EC, EGCG, and ECG were calculated as 1.13, 0.95, 0.68, and 0.34 for never a smoker and 0.10, 0.003, 0.17, and 0.04 for past smoker, respectively. With regard to the stability of risk estimates, when never smoker and past smoker were combined, the corresponding values were calculated as 1.09, 0.77, 0.70, and 0.50, respectively. On the other hand, the point estimates for current smoker consistently appeared to be ~2.00 although with no statistical significance; the corresponding values were calculated as 2.11, 1.64, 1.77, and 2.06, respectively. The interaction between cigarette smoking and tea polyphenols was also assessed. When compared with EGC = 0 and never or past smoker, the multivariate ORs of EGC = 0 and current smoker, EGC > 0 and never or past smoker, and EGC > 0 and current smoker were 1.5, 2.0, and 2.9, respectively. However, the interaction term did not reach the level of statistical significance ($P$ for interaction = 0.78). Similar results were observed for the other tea polyphenols.

## Discussion

An inverse association between plasma levels of tea polyphenols and gastric cancer was suggested in women but not in men. This observation is in line with our previous investigation, which was based on a cohort design in the same JPHC Study (21). In that study, an almost 30% reduction in gastric cancer risk was observed among women who drank five or more cups of green tea per day. No association was observed among men. Recent cohort studies that investigated green tea intake and gastric cancer risk concluded that there is no association between green tea intake and gastric cancer risk (22-24). Among these studies, Tsubono et al. (22) and Hoshiyama et al. (23) also showed that the results differed between men and women. Including our previous study, these studies agree that a reduced gastric cancer risk is suggested for only women (Table 5).

One explanation for the pattern between plasma tea polyphenols and gastric cancer differing by gender may be the modification effect of smoking. As shown in Table 3, the relationship between tea polyphenols and gastric cancer apparently showed a different pattern according to smoking status. Although the risk estimates did not reach the level of statistical significance owing to the small sample size, the contrast was quite apparent, and it may be reasonable to assume that these data suggest that cigarette smoking acts as a modifier of the green tea-gastric cancer relationship.

There is growing evidence that cigarette smoking modifies the chemopreventive efficacy of nutrient supplements (25). Two famous trials, the α-Tocopherol β-Carotene Cancer Prevention Study (26) and the Carotene and Retinol Efficacy Trial (27), revealed that smokers assigned to receive supplemental β-carotene had a statistically significantly higher lung cancer risk. Beyond these studies, interactions of chemopreventive agents with smoking have been reported in several observational or interventional studies. Some animal/mechanistic studies also support a biological interaction between high-dose β-carotene and tobacco exposure. Thus, it is not that far fetched to speculate that tea polyphenols reduce the risk of gastric cancer in nonsmokers and actually increase the risk of gastric cancer in smokers. Previously, few epidemiologic studies regarding green tea and gastric cancer risk have shown the data stratified by smoking status. Tsubono et al. (22) revealed a trend toward a positive association between green tea consumption and gastric cancer in subjects currently smoking 20 or more cigarettes per day but not in those currently smoking 1 to 19 cigarettes per day, those who smoked in the past, or those who had never smoked ($P$ for trend = 0.06). However, the interaction was not apparent ($P$ for interaction term = 0.17; ref. 22). To clarify this important issue, information from large studies is needed.

Another explanation for the different pattern between plasma levels of tea polyphenols and gastric cancer

**Table 1. Baseline characteristics of male controls according to serum tea polyphenol level (Cont'd)**

| Plasma EGCG level (ng/mL) | | | | Plasma ECG level (ng/mL) | | | |
|---|---|---|---|---|---|---|---|
| T1: 0 ($n$ = 205) | T2: 1.77-38.9 ($n$ = 62) | T3: 39.0+ ($n$ = 64) | $P$ for difference* | T1: 0 ($n$ = 253) | T2: 0.32-9.9 ($n$ = 38) | T3: 10.0+ ($n$ = 40) | $P$ for difference* |
| 59.2 (1.2) | 58.5 (1.1) | 56.5 (1.3) | 0.45 | 57.2 (0.8) | 58.5 (1.2) | 58.5 (1.5) | 0.65 |
| 38.5 | 48.4 | 54.7 | 0.05 | 40.7 | 47.4 | 57.5 | 0.12 |
| 70.2 | 69.4 | 65.6 | 0.78 | 70.8 | 68.4 | 60.0 | 0.39 |
| 39.0 | 37.1 | 46.9 | 0.46 | 41.1 | 36.8 | 37.5 | 0.82 |
| 5.50 (0.17) | 5.88 (0.30) | 4.96 (0.30) | 0.09 | 5.65 (0.15) | 4.94 (0.38) | 4.81 (0.37) | 0.04 |
| 42.0 | 46.8 | 34.4 | 0.36 | 41.9 | 36.8 | 42.5 | 0.83 |
| 46.3 | 38.7 | 39.1 | 0.41 | 44.7 | 44.7 | 35.0 | 0.51 |
| 51.2 | 64.5 | 56.3 | 0.18 | 54.2 | 55.3 | 57.5 | 0.92 |
| 48.8 | 74.2 | 70.3 | 0.0001 | 52.2 | 76.3 | 75.0 | 0.001 |
| 6.8 | 12.9 | 7.8 | 0.31 | 9.1 | 2.6 | 7.5 | 0.39 |
| 76.1 | 80.7 | 71.9 | 0.51 | 75.9 | 81.6 | 72.5 | 0.63 |
| 23.0 (0.5) | 23.0 (0.4) | 23.3 (0.5) | 0.9 | 23.2 (0.3) | 23.8 (0.5) | 22.2 (0.6) | 0.11 |

observed by gender may be that the increased risk of gastric cancer from smoking overwhelms the protective effect of tea polyphenols in men. According to the IARC, numerous epidemiologic studies (both cohort and case-control) conducted throughout the world have revealed a consistent association between cigarette smoking and gastric cancer (28). In fact, in our previous study based on a cohort design in the same JPHC Study (which investigated the effects of cigarette smoking on the risk of gastric cancer), we observed that the adjusted relative risk for past and current smokers was 1.6 and 1.7, respectively, compared with never smokers (29). In contrast, because the protective effect of green tea has been reported mainly at an intense dose such as 7 or more (30) or 10 or more (31) cups per day, the effect of green tea drinking may be slight.

Furthermore, prooxidant properties of tea polyphenols (32, 33) or other factors related to men may explain the unexpected findings observed in men.

A protective effect of tea polyphenols was suggested in women, although the result was significant for only ECG, for which only 27% of subjects reached the detection limit of measurement. Thus, the possibility of a chance finding cannot be completely ruled out. Furthermore, as presented in Table 2, the prevalence of *H. pylori* seropositivity declined sharply when the tea polyphenol level increased. We observed previously that *H. pylori* infection is related to a 5-fold increased risk of gastric cancer in the same data set, and *H. pylori* seropositivity undoubtedly plays an important role in gastric carcinogenesis (34). Although the protective effect of tea polyphenols remained even after *H. pylori* infection was adjusted, the reduced risk for women may be partly explained by the confounding effect of *H. pylori* infection. Some researchers used an animal model to report the inhibition of *H. pylori* urease by green tea extract (35) and the bactericidal effect of green tea catechins on *H. pylori* infection (36). 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. However, the reason why the gradual decrease in prevalence of *H. pylori* infection in relation to tea polyphenol level is seen in only women is unknown.

Pharmacokinetics of tea polyphenols in humans has been evaluated in several recent studies (37-39). Lee et al. (37) reported that plasma tea polyphenol levels peak 1 to 2 h after oral tea consumption and gradually reduce to undetectable levels in about 24 h on average in eight subjects. This finding is consistent with other studies (38, 39), which reported within-individual variability and considerable difference among individuals. This is the first epidemiologic study to investigate the association of plasma levels of tea polyphenols and gastric cancer risk. Therefore, we do not have information on the correlation between serum levels of tea polyphenols and self-reported green tea intake from other studies. For instance, in many previous studies, the correlation between dietary intakes and serum or plasma levels of α-carotene and β-carotene ranged from 0.25 to 0.53 and 0.15 to 0.36, respectively (40). In light of the low bioavailability of tea polyphenols, the observed correlation in our study may be reasonably good. Because most of our study subjects commonly consume tea almost daily, the one-point measurement of plasma polyphenol level may reproduce the tea drinking habit to some extent. Also, the fasting time at blood donation (time from last meal to blood donation) was matched between case and control, which may not cause a serious bias.

A large percentage of subjects was grouped as not having a detectable level of tea polyphenols because of the rather poor bioavailability and the detection limit of the measurement. Among male controls who reported that the frequency of green tea consumption was ''almost none,'' the plasma levels of EGC, EC, EGCG, and ECG were not detected for 81%, 87%, 90%, and 90%, respectively. The same percentage for women was 64%, 71%, 71%, and 79%, respectively. Japan is known as a country where green tea is widely consumed. In fact, only 9.6% of men and 9% of women reported their frequency of green tea intake as ''almost none.'' Even among a population with a high intake of green tea, a large percentage of subjects had no detectable plasma level of tea polyphenols. This finding means that we had a special opportunity to use a plasma biomarker to investigate the relationship between green tea and gastric cancer. The long storage time also may have lead to a large percentage of subjects having no detectable plasma level of tea polyphenols. We do not have data regarding the effect of storage time on plasma tea polyphenols. Blood samples of cases and controls were stored under the same conditions and had identical storage time

**Table 2. Baseline characteristics of female controls according to serum tea polyphenol level**

| | Plasma EGC level (ng/mL) | | | | Plasma EC level (ng/mL) | | | |
|---|---|---|---|---|---|---|---|---|
| | T1: 0 ($n$ = 80) | T2: 1.37-80.1 ($n$ = 42) | T3: 80.2+ ($n$ = 41) | $P$ for difference* | T1: 0 ($n$ = 97) | T2: 1.58-27.9 ($n$ = 34) | T3: 28.0+ ($n$ = 32) | $P$ for difference* |
| Age | 55.8 (2.3) | 63.1 (2.0) | 58.3 (2.3) | 0.04 | 56.1 (2.0) | 59.7 (1.5) | 61.3 (2.3) | 0.31 |
| Current smoking (%) | 2.5 | 2.4 | 9.8 | 0.14 | 2.1 | 2.9 | 12.5 | 0.04 |
| Alcohol consumption, 1+/wk (%) | 12.5 | 16.7 | 14.6 | 0.82 | 13.4 | 14.7 | 15.6 | 0.95 |
| Cod roe or fish gut, 1+d/wk (%) | 36.3 | 26.2 | 36.6 | 0.49 | 34.0 | 35.3 | 31.3 | 0.94 |
| Total salt (g/d) | 4.48 (0.21) | 3.91 (0.3) | 4.72 (0.3) | 0.14 | 4.41 (0.20) | 4.23 (0.33) | 4.52 (0.34) | 0.83 |
| Green-yellow vegetables, daily (%) | 57.5 | 59.5 | 48.8 | 0.56 | 59.8 | 50.0 | 50.0 | 0.47 |
| Other vegetables, 3+/wk (%) | 58.8 | 38.1 | 46.3 | 0.08 | 57.7 | 38.2 | 40.6 | 0.07 |
| Fruit, 3+/wk (%) | 82.5 | 64.3 | 82.9 | 0.047 | 80.4 | 64.7 | 84.4 | 0.1 |
| Green tea, 3+ cups/d (%) | 52.5 | 61.9 | 75.6 | 0.047 | 55.7 | 58.8 | 78.1 | 0.08 |
| Family history of gastric cancer (%) | 2.5 | 11.9 | 12.2 | 0.07 | 5.2 | 5.9 | 15.6 | 0.13 |
| *H. pylori* infection (%) | 78.8 | 69.1 | 63.4 | 0.17 | 78.4 | 58.8 | 68.8 | 0.08 |
| BMI | 23.6 (1.0) | 23.1 (0.8) | 24.0 (1.0) | 0.72 | 23.4 (0.8) | 23.2 (0.6) | 24.1 (0.9) | 0.65 |

NOTE: Values are means (SE), except otherwise specified.
*Based on $\chi^2$ test or ANOVA.

(blood donation date was a matching factor) and were tested in the same column to minimize the effect of measurement errors. Therefore, the possible degradation of tea polyphenols in plasma over time should not affect our conclusion with regard to case-control differences.

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, when compared with nonparticipants, participants in the health checkup survey, especially women, had a different socioeconomic status and a favorable lifestyle profile, such as smoking less, participating in more physical exercise, and eating more green vegetables or fruits (41). This finding means that caution is needed in generalizing or interpreting the results in this report.

The advantage of this study is its prospective design, which is free from several biases that are inevitable in a retrospective study. Other strengths include the following: the loss to follow-up was negligible, the quality of our cancer registry system was satisfactory during the study period, and potential confounding factors could be adjusted to minimize their influence on risk values. Furthermore, this is the first study to investigate the relationship between plasma levels of tea polyphenols and gastric cancer risk.

In conclusion, an inverse association between plasma tea polyphenols and gastric cancer risk was suggested among women, but no such association was seen for men. Furthermore, cigarette smoking was suggested to play a role as an effect modifier and explains in part the different patterns observed by gender. For a current smoker, can green tea intake be recommended? Our results showed that a past smoker had a similar or rather lower risk estimate than one who never smoked. Smoking cessation may be one of the important keys in

**Table 3. Matched OR (95% CI) of developing gastric cancer by baseline polyphenol levels in men and women**

| Men | | | Women | | |
|---|---|---|---|---|---|
| Tea polyphenol (ng/mL) | Case/control | Adjusted* OR (95% CI) | Tea polyphenol (ng/mL) | Case/control | Adjusted* OR (95% CI) |
| EGC | | | | | |
| T1: 0 | 136/167 | 1.0 | 0 | 79/80 | 1.0 |
| T2: 1.37-77.9 | 94/81 | 1.85 (1.15-2.97) | 1.37-80.1 | 50/42 | 1.81 (0.88-3.73) |
| T3: 78.0+ | 101/83 | 2.06 (1.23-3.45) | 80.2+ | 34/41 | 0.98 (0.45-2.15) |
| $P$ for trend | | 0.003 | | | 0.89 |
| EC | | | | | |
| T1: 0 | 180/200 | 1.0 | 0 | 106/97 | 1.0 |
| T2: 1.58-33.1 | 77/65 | 1.44 (0.91-2.28) | 1.58-27.9 | 31/34 | 0.78 (0.36-1.69) |
| T3: 33.2+ | 74/66 | 1.35 (0.80-2.30) | 28.0+ | 26/32 | 0.78 (0.37-1.62) |
| $P$ for trend | | 0.18 | | | 0.47 |
| EGCG | | | | | |
| T1: 0 | 180/205 | 1.0 | 0 | 111/99 | 1.0 |
| T2: 1.77-38.9 | 81/62 | 1.71 (1.06-2.75) | 1.77-36.2 | 32/32 | 1.19 (0.55-2.57) |
| T3: 39.0+ | 70/64 | 1.48 (0.89-2.44) | 36.3+ | 19/32 | 0.54 (0.25-1.16) |
| $P$ for trend | | 0.08 | | | 0.19 |
| ECG | | | | | |
| T1: 0 | 247/253 | 1.0 | 0 | 133/120 | 1.0 |
| T2: 0.32-9.9 | 37/38 | 1.00 (0.57-1.73) | 0.32-9.2 | 20/21 | 1.03 (0.41-2.59) |
| T3: 10.0+ | 47/40 | 1.22 (0.69-2.15) | 9.3+ | 10/22 | 0.25 (0.08-0.73) |
| $P$ for trend | | 0.55 | | | 0.02 |

*Matched for age, study area, date of blood donation, and fasting time at blood donation. Further adjusted for cigarette smoking, consumption of salted fish preserves, salt, fruit, green-yellow vegetables, other vegetables, BMI, family history of gastric cancer, and *H. pylori* infection.

**Table 2. Baseline characteristics of female controls according to serum tea polyphenol level (Cont'd)**

| Plasma EGCG level (ng/mL) | | | | Plasma ECG level (ng/mL) | | | |
|---|---|---|---|---|---|---|---|
| T1: 0 ($n$ = 99) | T2: 1.77-36.2 ($n$ = 32) | T3: 36.3+ ($n$ = 32) | $P$ for difference* | T1: 0 ($n$ = 120) | T2: 0.32-9.2 ($n$ = 21) | T3: 9.3+ ($n$ = 22) | $P$ for difference* |
| 62.3 (1.8) | 59.1 (1.8) | 55.7 (2.0) | 0.13 | 55.7 (1.0) | 59.3 (1.7) | 62.2 (2.2) | 0.02 |
| 1.0 | 12.5 | 6.3 | 0.02 | 3.3 | 4.8 | 9.1 | 0.47 |
| 12.1 | 15.6 | 18.8 | 0.62 | 13.3 | 23.8 | 9.1 | 0.34 |
| 35.4 | 28.1 | 34.4 | 0.75 | 35.0 | 23.8 | 36.4 | 0.58 |
| 4.45 (0.20) | 4.18 (0.34) | 4.44 (0.34) | 0.79 | 4.42 (0.17) | 3.60 (0.42) | 4.98 (0.41) | 0.06 |
| 59.6 | 53.1 | 46.9 | 0.43 | 60.8 | 33.3 | 50.0 | 0.05 |
| 57.6 | 43.8 | 34.4 | 0.05 | 55.8 | 23.8 | 45.5 | 0.02 |
| 76.8 | 78.1 | 81.3 | 0.87 | 80.8 | 61.9 | 77.3 | 0.16 |
| 52.5 | 65.6 | 81.3 | 0.01 | 59.2 | 57.1 | 72.7 | 0.46 |
| 5.1 | 9.4 | 12.5 | 0.33 | 4.2 | 14.3 | 18.2 | 0.03 |
| 79.8 | 68.8 | 53.1 | 0.01 | 77.5 | 57.1 | 59.1 | 0.05 |
| 22.9 (0.8) | 23.9 (0.7) | 23.8 (0.8) | 0.68 | 24.0 (0.4) | 23.6 (0.7) | 23.1 (0.9) | 0.64 |

gastric cancer prevention. The effect of green tea as a chemopreventive agent can be expected only in the absence of tobacco smoke exposure. Although the interpretations of the results are limited owing to the percentage of subjects whose detectable plasma levels of tea polyphenols was minimal, our results add important evidence to recent cohort studies in Japan in which different risk patterns by gender were observed.

## Appendix A. Members of the JPHC-based Prospective Study Group

The members of the JPHC-based Prospective Study Group are S. Tsugane, M. Inoue, T. Sobue, and T. Hanaoka (National Cancer Center, Tokyo); J. Ogata, S. Baba, T. Mannami, and A. Okayama (National Cardiovascular Center, Suita); K. Miyakawa, F. Saito, A. Koizumi, Y. Sano, and I. Hashimoto (Iwate Prefectural Ninohe Public Health Center, Ninohe); Y. Miyajima, N. Suzuki, S. Nagasawa, and Y. Furusugi (Akita Prefectural Yokote Public Health Center, Yokote); H. Sanada, Y. Hatayama, F. Kobayashi, H. Uchino, Y. Shirai, T. Kondo, R. Sasaki, Y. Watanabe, and Y. Miyagawa (Nagano Prefectural Saku Public Health Center, Saku); Y. Kishimoto, E. Takara, T. Fukuyama, M. Kinjo, M. Irei, and H. Sakiyama (Okinawa Prefectural Chubu Public Health Center, Okinawa); K. Imoto, H. Yazawa, T. Seo, A. Seiko, F. Ito, and F. Shoji (Katsushika Public Health Center, Tokyo); A. Murata, K. Minato, K. Motegi, and T. Fujieda (Ibaraki Prefectural Mito Public Health Center, Mito); K. Matsui, T. Abe, M. Katagiri, and M. Suzuki (Niigata Prefectural Kashiwazki and Nagaoka Public Health Center, Kashiwazaki and Nagaoka); M. Doi, A. Terao, and Y. Ishikawa (Kochi Prefectural Chuo-higashi Public Health Center, Tosayamada); H. Sueta, H. Doi, M. Urata, N. Okamoto, and F. Ide (Nagasaki Prefectural Kamigoto Public Health Center, Arikawa); H. Sakiyama, N. Onga, and H. Takaesu (Okinawa

**Table 4. Matched OR (95% CI) of developing gastric cancer by baseline tea polyphenol levels according to smoking status in men and women**

| Tea polyphenol (ng/mL) | Never smoker | | Past smoker | | Current smoker | |
|---|---|---|---|---|---|---|
| | Cases/controls | Adjusted* OR (95% CI) | Cases/controls | Adjusted* OR (95% CI) | Cases/controls | Adjusted* OR (95% CI) |
| EGC | | | | | | |
| T1 | 112/127 | 1.0 | 31/52 | 1.0 | 72/68 | 1.0 |
| T2 | 67/63 | 2.29 (1.10-4.80) | 33/23 | 11.17 (0.32-386.78) | 44/37 | 0.92 (0.25-3.43) |
| T3 | 48/52 | 1.13 (0.50-2.57) | 26/26 | 0.10 (0.001-7.48) | 61/46 | 2.11 (0.51-8.67) |
| $P$ for trend | | 0.54 | | 0.81 | | 0.26 |
| EC | | | | | | |
| T1 | 147/153 | 1.0 | 45/63 | 1.0 | 94/81 | 1.0 |
| T2 | 45/49 | 0.79 (0.35-1.81) | 29/17 | 0.30 (0.007-13.29) | 34/33 | 1.15 (0.37-3.57) |
| T3 | 35/40 | 0.95 (0.45-2.03) | 16/21 | 0.003 (0.000-2.30) | 49/37 | 1.64 (0.38-7.05) |
| $P$ for trend | | 0.82 | | 0.20 | | 0.51 |
| EGCG | | | | | | |
| T1 | 151/161 | 1.0 | 47/63 | 1.0 | 93/80 | 1.0 |
| T2 | 50/41 | 1.45 (0.66-3.17) | 25/19 | 1.84 (0.16-20.69) | 39/34 | 0.77 (0.23-2.56) |
| T3 | 26/40 | 0.68 (0.31-1.49) | 18/19 | 0.17 (0.006-4.55) | 45/37 | 1.77 (0.47-6.68) |
| $P$ for trend | | 0.57 | | 0.47 | | 0.43 |
| ECG | | | | | | |
| T1 | 112/127 | 1.0 | 64/76 | 1.0 | 128/107 | 1.0 |
| T2 | 67/63 | 1.13 (0.46-2.79) | 15/14 | 0.47 (0.01-21.48) | 16/19 | 0.17 (0.03-0.86) |
| T3 | 48/52 | 0.34 (0.11-0.99) | 11/11 | 0.04 (0.000-3.58) | 33/25 | 2.06 (0.51-8.29) |
| $P$ for trend | | 0.10 | | 0.19 | | 0.46 |

*Matched for age, study area, date of blood donation, and fasting time at blood donation. Further adjusted for cigarette smoking, consumption of salted fish preserves, salt, fruit, green-yellow vegetables, other vegetables, BMI, family history of gastric cancer, and *H. pylori* infection.

**Table 5. Summary of recent cohort studies investigating the relation between green tea intake and gastric cancer in Japan**

| Author (published year) | No. subjects | Follow-up period (y) | No. incident cases or death | Green tea intake (cups/d) | Adjusted relative risk (95% CI) | |
|---|---|---|---|---|---|---|
| | | | | | Men | Women |
| Tsubono et al. (22) | Men: 11,902/ women: 14,409 | 9 | Men: 296 cases/ women: 123 cases | <1 | 1.0 | 1.0 |
| | | | | 1-2 | 1.3 (0.8-1.9) | 0.8 (0.5-1.5) |
| | | | | 3-4 | 1.2 (0.8-1.8) | 0.7 (0.4-1.3) |
| | | | | 5+ | 1.5 (1.0-2.1) | 0.8 (0.5-1.3) |
| | | | | *P* for trend | 0.03 | 0.46 |
| Hoshiyama et al. (23) | Men: 30,370/ women: 42,481 | Men: 8 (mean)/ women: 8.2 (mean) | Men: 240 death/ women: 119 death | <1 | 1.0 | 1.0 |
| | | | | 1-2 | 1.6 (0.9-2.9) | 1.1 (0.5-2.5) |
| | | | | 3-4 | 1.1 (0.6-1.9) | 1.0 (0.5-2.1) |
| | | | | 5-9 | 1.1 (0.6-1.9) | 0.8 (0.4-1.6) |
| | | | | 10+ | 1.0 (0.5-2.0) | 0.7 (0.3-2.0) |
| | | | | *P* for trend | 0.63 | 0.48 |
| Sasazuki et al. (21) | Men: 34,832/ women: 38,111 | 12 | Men: 665 cases/ women: 227 cases | <1 | 1.0 | 1.0 |
| | | | | 1-2 | 0.9 (0.7-1.2) | 0.9 (0.5-1.4) |
| | | | | 3-4 | 0.8 (0.7-1.1) | 1.0 (0.7-1.6) |
| | | | | 5+ | 1.0 (0.8-1.3) | 0.7 (0.4-1.0) |
| | | | | *P* for trend | 0.65 | 0.08 |



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