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Urinary tea polyphenols in relation to gastric and esophageal cancers: a prospective study of men in Shanghai, China

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Experimental studies have shown that tea and tea polyphenols have anticarcinogenic properties. There have been no prospective investigations examining the relationship between tea polyphenols and cancer risk using validated biomarkers. In the present study, a nested case–control study design was used to investigate the association between prediagnostic urinary tea polyphenol markers and subsequent risk of gastric and esophageal cancers. One hundred and ninety incident cases of gastric cancer and 42 cases of esophageal cancer occurring in members of the Shanghai Cohort (18 244 men aged 45–64 years at recruitment with up to 12 years of follow-up) were compared with 772 cohort control subjects. The control subjects were individually matched to the index cases by age, month and year of sample collection, and neighborhood of residence (case–control ratio = 1:3 for gastric cancer, 1:5 for esophageal cancer). Urinary tea polyphenols, including epigallocatechin (EGC) and epicatechin (EC), and their respective metabolites 5-(3′,4′,5′-trihydroxyphenyl)-γ-valerolactone (M4) and 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone (M6), were measured in all study subjects by means of a validated assay. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated from logistic regression models. After exclusion of cases diagnosed under 4 years follow-up, urinary EGC positivity showed a statistically significant inverse association with gastric cancer (OR = 0.52, 95% CI = 0.28–0.97) after adjustment for *Helicobacter pylori* seropositivity, smoking, alcohol drinking, and level of serum carotenes. The protective effect was primarily seen among subjects with low (below population median) serum carotenes. The odds ratio for EGC positivity was 0.49 (95% CI = 0.26–0.94) among subjects with low serum carotenes while the corresponding odds ratio among subjects with higher levels of serum carotenes was 1.02 (95% CI = 0.46–2.28). Similar tea polyphenol–cancer risk associations were observed when the gastric cancer and esophageal cancer sites were combined. The present study provides direct evidence that tea polyphenols may act as chemopreventive agents against gastric and esophageal cancer development.

Abbreviations: CI, confidence interval; EC, epicatechin; EGC, epigallocatechin; EGCG, epigallocatechin gallate; M4, 5-(3′,4′,5′-trihydroxyphenyl)-γ-valerolactone; M6, 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone; OR, odds ratio.

Introduction

Gastric and esophageal cancers are the 2nd and 8th most common cancers worldwide (1). Both cancers display considerable variation in international incidence (1). China is a high risk country for both. In Shanghai, the age standardized incidence rate for gastric cancer is about 47 per 100 000 person-years in men and 21 per 100 000 person-years in women, which are 6–7 fold higher than comparable rates in USA whites (2). Similarly, the age standardized incidence rates for esophageal cancer in Shanghai men and women are 13 and 5 per 100 000 person-years, respectively. The corresponding rates in USA whites are 4 and 1 per 100 000 person-years (2).

Tea is one of the most popular beverages consumed around the world. There are two major categories of tea, distinguished by the varying methods of processing the tea leaves following harvest. In the processing of green tea, fresh tea leaves are steamed or dried at high temperature right after plucking, resulting in minimal oxidation of the naturally occurring polyphenols in the tea leaves. In contrast, fresh tea leaves are crushed during the manufacture of black tea to encourage oxidation and polymerization of the polyphenols in a fermentation process. In general, the amounts of polyphenols [primarily four catechins: epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin (EC) and epicatechin gallate] in green tea are ~30–40% of the weight of the water extractable materials, as compared with 3–10% in black tea (3). The bioavailability of tea catechins following tea consumption has been demonstrated. Plasma concentrations of EGCG, EGC, and EC reach peak levels between 1.5–2.5 h after tea consumption (4). Maximum urinary excretion of EGC and EC (mostly in the sulfate- and glucuronide-conjugated forms) normally occurs at 2–6 h after tea ingestion, and most is excreted within 8–9 h (4–6). On the other hand, maximum excretion of 5-(3′,4′,5′-trihydroxyphenyl)-γ-valerolactone (M4) and 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone (M6), which are the respective metabolites of EGC and EC, usually occurs between 7.5–13.5 h after tea intake (5). Cumulative excretion of EGC increased with increasing dose of green tea ingestion in human subjects (6). EGCG is not detectable in urine. There is now a validated assay for quantifying urinary EGC, EC, M4, and M6 in humans (4–7).

There are abundant *in vitro* and non-human *in vivo* data in support of the hypothesis that tea polyphenols are chemopreventive agents against both gastric and esophageal cancers (8–14). Human data are limited, but two interview-based case–control studies of gastric and esophageal cancers, both conducted in Shanghai, China, reported protective effects of green tea consumption in a dose-dependent manner (15,16).

The present study prospectively examined the association between urinary tea polyphenols (EGC, EC, M4 and M6) and risk of gastric and esophageal cancers among middle aged and older men in Shanghai, China. Frequent consumption of green tea and the relatively high incidence of gastric and esophageal

cancers among Shanghai Chinese, combined with the availability of prediagnostic urine samples in the Shanghai Cohort Study provided a special opportunity to test the hypothesis that tea polyphenols protect against the development of gastric and esophageal cancer, using validated urinary biomarkers.

Participants and methods

The design of the Shanghai Cohort Study has been described in detail elsewhere (17,18). Between January 1986 and September 1989, 18 244 men between 45 and 64 years of age living in four small geographically defined areas of the city of Shanghai were recruited to participate in a prospective study of diet and cancer (~80% of eligible subjects). Each participant was interviewed in person using a structured questionnaire to obtain demographic information, use of tobacco and alcohol, usual adult diet, and medical history. Information on tea consumption was not asked during the in-person interview. At completion of the interview, a 10 ml non-fasting blood sample and a single void (i.e. spot) urine sample were collected from each participant. The blood and urine samples were collected usually between 5 p.m. and 9 p.m. Blood samples were centrifuged for serum within 4 h after collection, and the serum specimens were stored at –70°C. The urine samples were processed after overnight storage at 4°C, and aliquots of the urine samples were frozen at –20°C until analysis. Follow-up of cancer occurrence and death have been conducted through annual recontacts with all surviving cohort members and routine review of reports from the population based Shanghai Cancer Registry and from the Shanghai Municipal Vital Statistics Office.

Gastric cancer cases occurring among cohort members until March 1998 and esophageal cancer cases occurring until September 1998 were eligible for the present study. There were 197 incident cases of gastric cancer and 46 incident cases of esophageal cancer. Three control subjects for each gastric cancer case and five control subjects for each esophageal cancer case were randomly chosen from all cohort members who were free of the specific cancer at the time of diagnosis of the index case. Control subjects were matched to index cases by age (±2 years), month and year of sample collection, and neighborhood of residence. Prior urinary biomarker measurements had led to depletion of urine specimens on a number of study subjects. These subjects were excluded from the present study. Matched control subjects were excluded if the index cancer case subject was excluded. For gastric cancer, seven cases (and their matched controls) and seven additional control subjects not matched to those seven cases were excluded for this reason. Similarly, for esophageal cancer, four cases (and their matched controls) and one additional control subject not matched to those four cases were excluded. In total, 190 gastric cancer cases (with 563 matched controls) and 42 esophageal cancer cases (with 209 matched controls) were included in the present analysis.

Urinary EGC, EC, M4 and M6 were measured according to the validated method of Yang *et al.* (4–6). In short, the thawed urine sample (50 µl) was mixed with 150 µl of a pH 6.8 sodium phosphate buffer, 20 µl of Vc-EDTA solution, and 20 µl of a mixture of β-glucuronidase (500 units) and sulfatase (40 units). The mixture was incubated at 37°C for 45 min. Samples were analyzed on a HPLC system consisting of an ESA Model 465 refrigerated autosampler, two ESA-580 dual-piston pumps, and an ESA 5500 coulochem electrode array system. Standard tea polyphenols were used to check the stability of the HPLC system. Urine samples with known amounts of tea polyphenols were included in all batches. The detection limits for urinary EGC, EC, M4 and M6 were 10 ng/ml (7). The within-day coefficients of variation for EGC and EC in the urine samples with known amounts of tea polyphenols were <10%, and the between-day coefficients of variation were <15%. Duplicate measurements were made for each urine sample. The mean of the duplicate measurements was assigned as the sample value. If the coefficient of variation of the duplicate measurements was ⩾15%, the urine sample was remeasured. For any given set of cases and their matched controls, the samples were tested in a single batch to minimize impact of possible inter-batch variation on the comparability between cases and controls. Laboratory personnel were blinded to the case/control status of study subjects.

Data were analyzed by standard matched set methods (19). Urinary tea polyphenols were expressed in units of urinary creatinine. The distributions of these urinary biomarkers were markedly skewed toward high values. Thus, formal statistical tests were performed on log-transformed values, and geometric means (as opposed to arithmetic means) are presented. Undetectable values were recoded to 5 ng/ml (one-half the detection limit). Correlations between the four urinary tea polyphenol biomarkers as well as the correlations between urinary tea polyphenols and serum carotenes were assessed using the Pearson's correlation coefficient (20). In the analysis of variance models used to compare urinary tea polyphenol levels between cancer cases and controls, the block design (each block representing one matched case–control set) was used (20). Conditional logistic regression models were used to examine the associations between urinary tea polyphenols and risk of gastric and esophageal cancer. The magnitude of the associations were measured by odds ratios (ORs), along with their corresponding 95% confidence intervals (CIs) and *P* values.

Table I. Risk factors for gastric and esophageal cancer

	Gastric cancer *n* (%)	Esophageal cancer *n* (%)	Controls *n* (%)
Total subject	190 (100.0)	42 (100.0)	772 (100.0)
Cigarette smoking			
Never	59 (31.1)	11 (26.2)	325 (42.1)
Ever[a]	131 (68.9)	31 (73.8)	447 (57.9)
Age at starting to smoke			
⩽20 years	35 (18.4)	13 (31.0)	104 (13.5)
20+ years	96 (50.5)	18 (42.8)	343 (44.4)
Weekly alcohol drinking			
No	96 (50.5)	14 (33.3)	447 (57.9)
Yes	94 (49.5)	28 (66.7)	325 (42.1)
1–28 drinks/week	66 (34.7)	18 (42.9)	277 (35.9)
29+ drinks/week	28 (14.8)	10 (23.8)	48 (6.2)
H.pylori serologic status[b]			
Negative	20 (11.0)	–	94 (17.6)
Positive	161 (89.0)[c]	–	441 (82.4)
Serum carotenes (µg/dl)			
⩽10.10[d]	108 (57.4)	29 (69.0)	383 (49.8)
>10.10	80 (42.6)	13 (31.0)	386 (50.2)

[a]Smoked at least one cigarette per day for 6 months or longer.
[b]Only gastric cancer cases and their matched control subjects were tested for *H.pylori* serologic status. Nine gastric cancer cases and 28 control subjects with missing *H.pylori* serologic status were excluded.
[c]The seropositivity rate was 85.4% in cases diagnosed <5 years after enrollment and 92.9% in cases diagnosed 5 or more years after enrollment.
[d]Median value among all control subjects. Two gastric cancer cases and three control subjects with missing values on serum carotenes were excluded.

In the present study, the following factors were statistically significantly related to gastric or esophageal cancer risk in univariate analysis: cigarette smoking, alcohol drinking, level of serum carotenes (alpha- and beta-carotene combined), and positivity for *Helicobactor pylori* antibodies in serum (for gastric cancer only) (Table I). Multivariate stepwise regression analysis showed cigarette smoking, alcohol drinking, level of serum carotenes, and *H.pylori* seropositivity as independent risk factors for gastric cancer. Similarly, multivariate stepwise regression analysis showed cigarette smoking and alcohol drinking as independent risk factors for esophageal cancer. The independent risk factors for both cancers combined were found to be cigarette smoking, alcohol drinking and level of serum carotenes. These potential confounding variables were included in the multivariate conditional logistic regression models when we examined the associations between urinary tea polyphenols and risk of gastric and esophageal cancers.

We also examined the urinary tea polyphenol–cancer association stratified by cigarette smoking, alcohol drinking and level of serum carotenes. There are too few *H.pylori* seronegative subjects (seven cases) to allow for a meaningful analysis of the tea polyphenol–cancer association by serum *H.pylori* status. For the subgroup analyses, matched sets were broken and the unconditional logistic regression models involving all control subjects were used to maximize the number of subjects available for analysis. An indicator variable denoting separate laboratory batches was included in the unconditional logistic regression model to account for possible inter-batch variation. Parallel analyses with and without adjustment for the following factors—neighborhood of residence, month and year of sample collection, and, for gastric cancer, *H.pylori* seropositivity—were performed. Results of the parallel analyses were similar. Figures presented in Table V are based on analyses without adjustment for neighborhood of residence, month and year of sample collection, and *H.pylori* seropositivity.

Statistical computing was conducted using the SAS (SAS Institute Inc., NC) version 8.0 and Epilog windows version (Epicenter Software, CA) statistical software packages. Two-sided *P* values that are <0.05 and 95% CIs that do not include one are considered to be statistically significant. All *P* values quoted are 2-sided.

Table II. The Pearson's product–moment correlation coefficients between log-transformed urinary EGC, EC, M4, M6 (mg/g creatinine) and serum carotenes (μg/dl) among control subjects ($n = 772$)

	EGC	EC	M4	M6
EC	0.68[a]			
M4	0.41[a]	0.41[a]		
M6	0.44[a]	0.48[a]	0.83[a]	
Serum carotenes[b]	0.015	0.022	–0.015	0.003
Age	0.03	0.02	–0.03	–0.02
Ethanol intake (g/day)	0.13[a]	0.07[a]	0.01	0.03
Cigarettes/day	0.15[a]	0.09[a]	0.11[a]	0.08[a]

[a]$P < 0.05$.
[b]Three subjects with missing serum carotenes were excluded.

Results

Of the 190 gastric cancer patients, 45 (24%) had cardia cancer, 115 (60%) had non-cardia cancer, and 30 (16%) had unknown subsites. One hundred and seventy one (90%) gastric cancer patients were histologically confirmed. Of the 42 esophageal cancer patients, 28 (67%) had squamous cell carcinoma, 2 (5%) had adenocarcinoma, and 12 (28%) had other and unspecified types. Thirty-two (76%) esophageal cancer patients were histologically confirmed. The mean ages of gastric and esophageal cancer patients at diagnosis were 63.4 and 62.5 years, respectively. The corresponding figures for controls at the time of cancer diagnosis of the index cases were 63.3 and 62.6 years, respectively. The average time interval between urine sample collection and cancer diagnosis was 5.0 years for gastric cancer patients (range, 1 month–12 years) and 5.1 years for esophageal cancer patients (range, 2 months–11 years).

Eighty percent of all control subjects were positive for urinary EGC. The corresponding figures for urinary EC, M4 and M6 were 85%, 60% and 80%, respectively. Among all control subjects, the geometric means of EGC, EC, M4 and M6 were 0.37, 0.45, 0.39 and 0.76 mg/g creatinine, respectively. The comparable figures for gastric/esophageal cancer cases were 0.36, 0.47, 0.35 and 0.79 mg/g creatinine, respectively. There were no differences between cancer cases and controls (all $P > 0.40$). For *H.pylori* seronegative control subjects, the geometric means of EGC, EC, M4 and M6 were 0.38, 0.53, 0.47 and 0.96 mg/g creatinine, respectively. The comparable figures for *H.pylori* seropositive control subjects were 0.39, 0.51, 0.39 and 0.86 mg/g creatinine, respectively.

Table II presents product–moment correlation coefficients among the four urinary tea polyphenol biomarkers. The strongest correlation was noted between M4 and M6, followed by that between EGC and EC. There were scant correlations between any of the four urinary biomarkers and serum carotenes (Table II). There was no correlation between any of the urinary biomarkers and age at baseline interview. Urinary EGC and EC, but not M4 and M6, were positively correlated with alcohol drinking. All four urinary biomarkers were positively correlated with cigarette smoking (Table II).

Overall, there was no association between risk of gastric or esophageal cancer and positivity for (or level of) any of the four urinary tea polyphenols. Comparable results were obtained after adjustment for potential confounders (Table III). Similarly, analysis of variance tests comparing levels of urinary tea polyphenols between cases and their matched controls revealed no significant differences in the two groups (data not shown).

Table IV presents the urinary tea polyphenol–cancer associations by duration of follow-up. Since risks were comparable among subjects positive for any of the given urinary tea polyphenols, analysis was simplified to negative versus positive status for each of the four urinary biomarkers. Similarly, since all the urinary tea polyphenol–risk associations were comparable between gastric and esophageal cancers, the two sites were combined to yield more stable risk estimates since individual sample sizes, especially for esophageal cancer, became rather small after stratification. For EC, M4 and M6, all biomarker–risk associations remained null regardless of duration of follow-up. In contrast, EGC displayed an evident inverse association with risk of gastric cancer alone or gastric and esophageal cancer combined as duration of follow-up lengthened (Table IV). For cases diagnosed at least 4 years after enrollment, risk was significantly reduced by 48% (OR = 0.52, 95% CI = 0.28–0.97) for gastric cancer alone and 42% (OR = 0.58, 95% CI = 0.34–0.98) for gastric and esophageal cancer combined. For esophageal cancer alone, the reduction in risk associated with EGC positivity was roughly 40% (OR = 0.58, 95% CI = 0.19–1.79). As in the cases with gastric cancer, EC, M4 and M6 were unrelated to esophageal cancer risk (data not shown). For gastric cancer, the effect of EGC was further examined by subsite. We noted a slightly stronger inverse association for cardia (OR = 0.22, 95% CI = 0.05–0.90) than for non-cardia cancer (OR = 0.73, 95% CI = 0.38–1.41). However, the difference between these two odds ratios was not statistically significant ($P = 0.13$).

We examined the potential modifying effects of cigarette smoking, alcohol drinking, and serum carotenes on the EGC–cancer associations. Cancer cases with <4 years of follow-up were excluded since the data suggested that biomarker values obtained close to the time of cancer diagnosis might not be informative. Table V shows a stronger inverse association between EGC and cancer risk in never versus ever cigarette smokers, non- versus regular drinkers of alcohol, and those with low (i.e. below population median value) versus higher levels of serum carotenes. The differences in ORs between never and ever smokers, and between non- and regular drinkers of alcohol were not statistically significant (Table V). On the other hand, a borderline statistically significant interaction between EGC and serum carotene level on cancer risk was observed (Table V). Statistically significant inverse EGC–risk association was observed in those with low levels of serum carotenes (OR = 0.49, 95% CI = 0.26–0.94 for gastric cancer alone; OR = 0.46, 95% CI = 0.26–0.84 for gastric and esophageal cancer combined), but no such association was noted among subjects with higher serum carotene levels (OR = 1.02, 95% CI = 0.46–2.28 for gastric cancer alone; OR = 1.02, 95% CI = 0.47–2.21 for gastric and esophageal cancer combined). For esophageal cancer alone, an inverse, statistically non-significant association between risk and EGC positivity was observed in individuals with below median serum carotene levels (OR = 0.44, 95% CI = 0.14–1.35), but not in those with higher serum carotene levels (OR = 0.99, 95% CI = 0.10–10.05).

We repeated all of the analyses on histologically confirmed cases and their matched controls (203 cases and 669 controls). Results were similar to those based on the whole data set (data not shown).

Discussion

To our knowledge, this is the first epidemiologic study attempting to establish a direct link between prediagnostic tea

Table III. Urinary EGC, EC, M4, and M6 levels (mg/g creatinine) in relation to gastric and esophageal cancer risk

	Gastric cancer				Esophageal cancer			
	No. cases	No. controls	OR[a] (95% CI)	Adjusted OR[b] (95% CI)	No. cases	No. controls	OR[a] (95% CI)	Adjusted OR[c] (95% CI)
EGC								
Negative	40	102	1.00	1.00	10	55	1.00	1.00
Positive	150	461	0.81 (0.51–1.28)	0.73 (0.45–1.18)	32	154	1.14 (0.52–2.50)	0.87 (0.38–2.02)
⩽0.196[d]	65	224	0.70 (0.42–1.17)	0.64 (0.37–1.09)	13	81	0.87 (0.35–2.16)	0.84 (0.32–2.19)
0.197+	85	237	0.90 (0.55–1.47)	0.82 (0.49–1.38)	19	73	1.44 (0.61–3.37)	0.91 (0.36–2.31)
P for trend			0.97	0.77			0.34	0.86
EC								
Negative	17	72	1.00	1.00	7	47	1.00	1.00
Positive	173	491	1.60 (0.88–2.92)	1.74 (0.93–3.28)	35	162	1.49 (0.60–3.69)	1.22 (0.48–3.10)
⩽0.311[d]	71	203	1.56 (0.83–2.93)	1.71 (0.88–3.32)	24	113	1.46 (0.57–3.74)	1.32 (0.51–3.45)
0.312+	102	288	1.64 (0.88–3.07)	1.78 (0.92–3.44)	11	49	1.54 (0.53–4.45)	0.99 (0.32–3.04)
P for trend			0.20	0.17			0.46	0.91
M4								
Negative	73	222	1.00	1.00	16	87	1.00	1.00
Positive	117	341	1.06 (0.73–1.55)	1.06 (0.71–1.59)	26	122	1.16 (0.59–2.29)	0.91 (0.44–1.89)
⩽0.220[d]	55	150	1.13 (0.73–1.76)	1.13 (0.71–1.81)	17	78	1.19 (0.57–2.49)	0.96 (0.43–2.15)
0.220+	62	191	1.00 (0.65–1.54)	1.00 (0.63–1.58)	9	44	1.11 (0.45–2.75)	0.83 (0.32–2.14)
P for trend			0.99	0.97			0.75	0.71
M6								
Negative	23	82	1.00	1.00	15	74	1.00	1.00
Positive	167	481	1.31 (0.77–2.25)	1.52 (0.84–2.73)	27	135	0.99 (0.50–1.97)	0.79 (0.38–1.66)
⩽0.448[d]	76	217	1.32 (0.75–2.33)	1.55 (0.84–2.88)	18	87	1.03 (0.49–2.18)	0.90 (0.40–2.01)
0.449+	91	264	1.30 (0.74–2.30)	1.48 (0.79–2.76)	9	48	0.93 (0.38–2.28)	0.61 (0.22–1.65)
P for trend			0.49	0.40			0.89	0.35

[a]Matched odds ratios (ORs) with matching factors being: age, month and year of sample collection, and neighborhood of residence.
[b]Further adjusted for *H.pylori* seropositivity, age at starting to smoke (never, 20+ years, <20 years), number of alcoholic drinks per week (none, 1–28, 29+), and level of serum carotenes (in quartiles). Ten gastric cancer cases and 47 controls with missing *H.pylori* seropositivity or serum carotene values were excluded from the analysis.
[c]Further adjusted for age at starting to smoke (never, 20+ years, <20 years), and number of alcoholic drinks per week (none, 1–28, 29+).
[d]Median positive value among all control subjects.

Table IV. Urinary EGC, EC, M4 and M6 in relation to gastric/esophageal cancer risk by time interval between urine sample collection and cancer diagnosis

	Gastric cancer[a]				Gastric and esophageal cancer[b]			
	<4 years		4+ years		<4 years		4+ years	
	Ca/Co[c]	OR[d] (95% CI)	Ca/Co	OR[d] (95% CI)	Ca/Co	OR[e] (95% CI) Ca/Co	OR[e] (95% CI)	
Total subjects	79/217		101/299		104/347		126/416	
EGC								
Negative	8/27	1.00	31/68	1.00	11/53	1.00	38/100	1.00
Positive	71/190	1.25 (0.52–3.02)	70/231	0.52 (0.28–0.97)	93/294	1.40 (0.67–2.91)	88/316	0.58 (0.34–0.98)
EC								
Negative	0/16		16/53	1.00	2/27	1.00	21/90	1.00
Positive	79/201	–[f]	85/246	1.13 (0.55–2.30)	102/320	3.92 (0.92–16.71)	105/326	1.22 (0.67–2.23)
M4								
Negative	34/83	1.00	34/121	1.00	43/129	1.00	44/177	1.00
Positive	45/134	0.80 (0.45–1.43)	67/178	1.50 (0.82–2.75)	61/218	0.84 (0.52–1.37)	82/239	1.21 (0.74–2.00)
M6								
Negative	5/20	1.00	15/59	1.00	12/53	1.00	25/102	1.00
Positive	74/197	1.48 (0.52–4.17)	86/240	1.34 (0.63–2.82)	92/294	1.19 (0.59–2.41)	101/314	0.93 (0.52–1.67)

[a]Ten gastric cancer cases and 47 controls with missing *H.pylori* seropositivity or serum carotenes values were excluded from the analysis.
[b]Two gastric cancer cases and nine controls with missing serum carotene values were excluded from the analysis.
[c]Number of cases/number of controls.
[d]Matched ORs with matching factors being: age, month and year of sample collection, and neighborhood of residence. Further adjusted for *H.pylori* seropositivity, age at starting to smoke (never, 20+ years, <20 years), number of alcoholic drinks per week (none, 1–28, 29+), and level of serum carotenes (in quartiles).
[e]In addition to matching factors, matched ORs were further adjusted for age at starting to smoke (never, 20+ years, <20 years), number of alcoholic drinks per week (none, 1–28, 29+), and level of serum carotenes (in quartiles).
[f]There was no case in the reference category, thus, no finite estimate for OR could be calculated.

Table V. Urinary EGC and gastric/esophageal cancer risk stratified by levels of cigarette smoking, alcohol drinking, and serum carotenes; cancer cases with <4 years of follow-up are excluded

		Gastric cancer		Gastric and esophageal cancer	
	No. controls[a]	No. cases[b]	OR (95% CI)	No. cases[b]	OR (95% CI)
Cigarette smoking					
Never					
EGC negative	78	12	1.00	13	1.00
EGC positive	246	15	0.45 (0.19–1.03)[c]	18	0.48 (0.21–1.07)[c]
Ever					
EGC negative	77	19	1.00	25	1.00
EGC positive	368	58	0.82 (0.45–1.51)[c]	70	0.76 (0.43–1.32))[c]
Weekly alcohol drinking					
No					
EGC negative	95	16	1.00	19	1.00
EGC positive	350	33	0.56 (0.28–1.11)[d]	35	0.54 (0.28–1.04)[d]
Yes					
EGC negative	60	15	1.00	19	1.00
EGC positive	264	40	0.77 (0.38–1.56)[d]	53	0.76 (0.40–1.44)[d]
Serum carotenes (μg/dl)					
≤10.10[e]					
EGC negative	79	22	1.00	28	1.00
EGC positive	304	33	0.49 (0.26–0.94)[f]	43	0.46 (0.26–0.84)[f]
>10.10					
EGC negative	76	9	1.00	10	1.00
EGC positive	310	40	1.02 (0.46–2.28)[f]	45	1.02 (0.47–2.21)[f]

[a]Seven hundred and sixty-nine controls were included in these analyses after excluding those with missing values on serum carotenes.
[b]One hundred and four gastric cancer cases and 22 esophageal cancer cases were included in these analyses after excluding those with missing values on serum carotenes.
[c]Unmatched ORs were adjusted for laboratory batch, age (continuous), number of alcoholic drinks per week (none, 1–28, 29+), and level of serum carotenes (in quartiles). *P* values for interaction between EGC and smoking were 0.34 for gastric cancer alone, and 0.53 for gastric and esophageal cancer combined.
[d]Unmatched ORs were adjusted for laboratory batch, age (continuous), age at starting to smoke (never, 20+ years, <20 years), and level of serum carotenes (in quartiles). *P* values for interaction between EGC and alcohol drinking were 0.80 for gastric cancer alone, and 0.57 for gastric and esophageal cancer combined.
[e]Median value among all control subjects.
[f]Unmatched ORs were adjusted for laboratory batch, age (continuous), number of alcoholic drinks per week (none, 1–28, 29+), and age at starting to smoke (never, 20+ years, <20 years). *P* value for interaction between EGC and level of serum carotenes were 0.07 for gastric cancer alone, and 0.057 for gastric and esophageal cancer combined.

polyphenol levels and subsequent risk of gastric/esophageal cancer. Although we found no associations between urinary tea polyphenols and gastric/esophageal cancer risk when all study subjects were considered, we noted evident inverse associations between one such polyphenol, EGC, and risk of gastric/esophageal cancer as duration of follow-up lengthened. There was a statistically significant 48% reduction in risk associated with EGC positivity when gastric cancer cases were restricted to those with at least 4 years of follow-up. Similarly, risk was reduced by 42% when gastric and esophageal cancer cases combined were restricted to those diagnosed 4 or more years post-enrollment. It is probable that some patients experienced increasingly severe clinical symptoms as their cancer advanced, thus possibly rendering the data on those enrolled in the cohort study relatively close to their dates of cancer diagnosis inappropriate for dietary studies. The present study suggests that the protective effect of tea polyphenols is somewhat more important among nonsmokers and nondrinkers of alcohol, but especially among individuals who are relatively deficient in carotenes.

A number of epidemiologic studies conducted in diverse populations have investigated the effect of tea consumption on gastric/esophageal cancer development. Among 12 studies from China and Japan, where green tea is preferred, seven of them have suggested a protective effect of green tea consumption on gastric/esophageal cancer risk, while four other studies failed to observe a protective effect (15,16,21–30). In the last study that used hospital patients as control subjects, the authors actually noted a significant increased risk of gastric cancer with green tea consumption (29). However, the prevalence of green tea drinking among the hospital control subjects was only 1.7% (29). In a population-based case–control study in Shanghai, China, involving 711 cases of gastric cancer and an equal number of age- and gender-matched population controls, a significant protective effect of green tea intake on gastric cancer risk was observed (OR = 0.71, 95% CI = 0.54–0.93) (16). The risk of gastric cancer decreased with increasing amount of green tea consumed daily (*P* for trend = 0.006) (16). In Japan, Kono *et al.* reported a relative risk of 0.3 (95% CI = 0.1–0.7) for gastric cancer in subjects who consumed >10 cups of green tea per day relative to those who consumed less (30). On the other hand, two recent prospective studies from Japan both failed to observe a protective effect of green tea drinking on gastric cancer risk (23,24). In the first study, green tea consumption was solicited from a self-administered questionnaire. Eighty-one percent of subjects reported drinking green tea daily, while only 5% subjects were not regular drinkers of green tea (24). The second Japanese study conducted on atomic bomb survivors also showed null results (23). The very high prevalence of relatively heavy drinkers of green tea coupled with the very low prevalence of non-exposed individuals among Japanese might be the reason for the two

studies' null findings. In other words, most of the exposure levels among study subjects might be outside the range within which the association between intake and cancer risk is most evident. Finally, in a population-based case–control study of esophageal cancer in Shanghai (902 cases and 1552 controls), green tea drinking was significantly associated with decreased risk of esophageal cancer in both men (OR = 0.43, 95% CI = 0.22–0.86) and women (OR = 0.40, 95% CI = 0.20–0.77). Importantly, this effect was confined to those who neither smoked cigarettes nor drank alcoholic beverages regularly (15). In the present study, we also observed a stronger effect of EGC positivity on cancer risk in subjects who never smoked cigarettes or never drank alcoholic beverages regularly.

In contrast, studies conducted in Western populations in which black tea is commonly consumed have yielded largely negative results on this relationship. Among the five population-based case–control and cohort studies (31–35) that have examined the tea–gastric cancer relationship, only one showed a significant inverse association (OR = 0.63, 95% CI = 0.43–0.91) (32). A probable explanation for this discrepancy in results between Asian and Western populations is that the levels of tea catechins are 3–10 times higher in green than black tea (3).

Experimental animal studies lend support to the hypothesis that green tea or tea polyphenols are chemopreventive agents for chemically induced gastric and esophageal cancer. Administration of green tea before and during carcinogen treatment significantly reduces incidence and multiplicity of forestomach tumors in mice (11). Similarly, esophageal tumor incidence is significantly reduced in carcinogen-treated rats given black or green tea (12). Experiments involving specific tea polyphenols have yielded comparable results (13,14).

Among the four urinary measurements of tea polyphenols (EGC, EC, M4 and M6) examined in the present study, EGC is a more direct biomarker of tea drinking. EC is not a specific marker of tea consumption as there are other common dietary sources such as apples, wine and chocolate (36,37); levels of M4 and M6, which are the respective metabolites of EGC and EC, are influenced by the microflora environment in the large intestine (5).

In the present study, we have noted differential effects of EGC and EC on gastric and esophageal cancer risk. While EGC showed a statistically significant protective effect against cancer risk once subjects with short follow-up were excluded from the analysis, no such association was observed with EC. This finding has supportive data from *in vitro* studies. It has been shown that EGC, but not EC, inhibits growth of human gastric cancer cell lines (10). There are no *in vivo* animal data on the potential anticarcinogenic effects of EGC versus EC. It is also possible that EGC is a surrogate of other as yet unidentified constituents in green tea that exert a protective effect on the development of gastric and esophageal cancers.

The anticarcinogenic activity of tea polyphenols is believed to be closely related to their antioxidative properties (38). Our observation that the inverse tea polyphenol–cancer association was largely confined to subjects relatively deficient in another class of antioxidants, i.e. the carotenes, tends to support this view. From our point of view, the null association between green tea drinking and risk of gastric cancer in the recent Japanese study published in the *New England Journal of Medicine* could partly be explicable by the subjects' relatively high level of fruit and vegetable intake (thus, high intake of carotenes) (24). In fact, the mean level of serum carotenes in male Japanese (32.3 μg/dl, calculated based on authors' reported value of 0.603 μmol/l) is considerably higher than that in our study population (10.1 μg/dl) (39). In addition, tea has been shown to inhibit endogenous nitrosation in humans (40,41); endogenously formed nitrosamines are recognized etiologic agents for gastric and esophageal cancer (42,43).

The present study has certain limitations. (i) The Shanghai Cohort Study, by design, relied on biomarkers for dietary exposure assessment. We did not collect information on tea consumption via in-person interviews at recruitment. Therefore, the present study cannot examine the relationship between self-reported tea consumption level and cancer risk, or the associations between levels of tea drinking and the four urinary tea polyphenols. (ii) A 24-h urine specimen would have offered a more stable estimate of urinary levels of tea polyphenols for each study subject. Unfortunately, this is impractical and too costly for a large-scale cohort study such as ours. However, there is indirect evidence that the spot urine specimens collected as part of the Shanghai Cohort Study are capable of yielding informative urinary biomarkers in exposure–cancer association studies. Previously, using aflatoxin metabolites detected from the single void urine samples, the Shanghai Cohort Study provided the first set of analytical human evidence linking aflatoxin exposure to hepatocellular carcinoma risk (17). Nonetheless, the large variability of measurements based on single void urine samples might explain the lack of a dose–response relationship between EGC and cancer risk in the present study. (iii) Another concern is the possible degradation of the test compounds in urine over time. No data are available regarding the effect of long term storage on the concentrations of urinary tea polyphenols, although prior study has shown that these urinary measurements remained unchanged when samples had been stored at –80 °C for 6 months (4). However, possible degradation of test compounds in urine over time should not affect our conclusion concerning case–control differences. Our cancer cases and their individually matched control subjects possessed identical storage time (a matching factor). Also, cancer cases and their matched controls were tested in a single batch to minimize the effect of possible inter-batch variation in measurement errors.

Although this study was based on a male cohort, there is no reason to doubt that the result would not be applicable to women. In fact, two interview-based case–control studies from Shanghai, China, have reported a stronger protective effect of green tea consumption among female subjects (15,16). Western populations consumed mainly black tea, which contains much less tea catechins than green tea. The finding of an inverse EGC–cancer risk association from the present study, if confirmed, would suggest that sufficient amounts of black tea should also yield a protective effect on gastric/esophageal cancer.

In conclusion, the present study, which is the first prospective study to evaluate biomarkers of tea polyphenol exposure and cancer risk, provides direct evidence that tea polyphenols may act as chemopreventive agents against gastric and esophageal cancer development.

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References

1. Parkin,D.M., Pisani,P. and Ferlay,J. (1999) Global cancer statistics. *CA Cancer J. Clin*., **49**, 33–64.
2. Parkin,D., Whelan,S.M., Ferlay,J., Raymond,L. and Young,J. (1997) *Cancer Incidence in Five Continents, Vol. VII*. IARC Scientific Publications, No. 143. Lyon.
3. Balentine,D.A., Wiseman,S.A. and Bouwens,L.C. (1997) The chemistry of tea flavonoids. *Crit. Rev. Food Sci. Nutr.*, **37**, 693–704.
4. Lee,M.J., Wang,Z.Y., Li,H., Chen,L., Sun,Y., Gobbo,S., Balentine,D.A. and Yang,C.S. (1995) Analysis of plasma and urinary tea polyphenols in human subjects. *Cancer Epidemiol. Biomarkers Prev*., **4**, 393–399.
5. Li,C., Lee,M.J., Sheng,S., Meng,X., Prabhu,S., Winnik,B., Huang,B., Chung,J.Y., Yan,S., Ho,C.T. and Yang,C.S. (2000) Structural identification of two metabolites of catechins and their kinetics in human urine and blood after tea ingestion. *Chem. Res. Toxicol*., **13**, 177–184.
6. Yang,C.S., Chen,L., Lee,M.J., Balentine,D., Kuo,M.C. and Schantz,S.P. (1998) Blood and urine levels of tea catechins after ingestion of different amounts of green tea by human volunteers. *Cancer Epidemiol. Biomarkers Prev*., **7**, 351–354.
7. Lee,M.J., Prabhu,S., Meng,X., Li,C. and Yang,C.S. (2000) An improved method for the determination of green and black tea polyphenols in biomatrices by high-performance liquid chromatography with coulometric array detection. *Anal. Biochem*., **279**, 164–169.
8. Chen,J. (1992) The effects of Chinese tea on the occurrence of esophageal tumors induced by N-nitrosomethylbenzylamine in rats. *Prev. Med*., **21**, 385–391.
9. Katiyar,S.K., Agarwal,R., Zaim,M.T. and Mukhtar,H. (1993) Protection against N-nitrosodiethylamine and benzo[*a*]pyrene-induced forestomach and lung tumorigenesis in A/J mice by green tea. *Carcinogenesis*, **14**, 849–855.
10. Okabe,S., Ochiai,Y., Aida,M., Park,K., Kim,S.J., Nomura,T., Suganuma,M. and Fujiki,H. (1999) Mechanistic aspects of green tea as a cancer preventive: effect of components on human stomach cancer cell lines. *Jpn. J. Cancer Res.*, **90**, 733–739.
11. Wang,Z.Y., Hong,J.Y., Huang,M.T., Reuhl,K.R., Conney,A.H. and Yang,C.S. (1992) Inhibition of N-nitrosodiethylamine- and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced tumorigenesis in A/J mice by green tea and black tea. *Cancer Res.*, **52**, 1943–1947.
12. Wang,Z.Y., Wang,L.D., Lee,M.J., Ho,C.T., Huang,M.T., Conney,A.H. and Yang,C.S. (1995) Inhibition of N-nitrosomethylbenzylamine-induced esophageal tumorigenesis in rats by green and black tea. *Carcinogenesis*, **16**, 2143–2148.
13. Yamane,T., Takahashi,T., Kuwata,K., Oya,K., Inagake,M., Kitao,Y., Suganuma,M. and Fujiki,H. (1995) Inhibition of N-methyl-N′-nitro-N-nitrosoguanidine-induced carcinogenesis by (−)-epigallocatechin gallate in the rat glandular stomach. *Cancer Res.*, **55**, 2081–2084.
14. Katiyar,S.K., Agarwal,R. and Mukhtar,H. (1993) Protective effects of green tea polyphenols administered by oral intubation against chemical carcinogen-induced forestomach and pulmonary neoplasia in A/J mice. *Cancer Lett*., **73**, 167–172.
15. Gao,Y.T., McLaughlin,J.K., Blot,W.J., Ji,B.T., Dai,Q. and Fraumeni,J.F.,Jr. (1994) Reduced risk of esophageal cancer associated with green tea consumption. *J. Natl Cancer Inst.*, **86**, 855–858.
16. Yu,G.P., Hsieh,C.C., Wang,L.Y., Yu,S.Z., Li,X.L. and Jin,T.H. (1995) Green-tea consumption and risk of stomach cancer: a population-based case–control study in Shanghai, China. *Cancer Causes Control*, **6**, 532–538.
17. Ross,R.K., Yuan,J.M., Yu,M.C., Wogan,G.N., Qian,G.S., Tu,J.T., Groopman,J.D., Gao,Y.T. and Henderson,B.E. (1992) Urinary aflatoxin biomarkers and risk of hepatocellular carcinoma. *Lancet*, **339**, 943–946.
18. Yuan,J.M., Ross,R.K., Wang,X.L., Gao,Y.T., Henderson,B.E. and Yu,M.C. (1996) Morbidity and mortality in relation to cigarette smoking in Shanghai, China. A prospective male cohort study. *JAMA*, **275**, 1646–1650.
19. Breslow,N.E. and Day,N.E. (1980) *Statistical Methods in Cancer Research: The Analysis of Case–control Studies*. IARC Scientific Publications, No. 32. Lyon.
20. Winer,B. (1971) *Statistical Principles in Experimental Design*. McGraw-Hill, New York.
21. Gao,C.M., Takezaki,T., Ding,J.H., Li,M.S. and Tajima,K. (1999) Protective effect of allium vegetables against both esophageal and stomach cancer: a simultaneous case-referent study of a high-epidemic area in Jiangsu Province, China. *Jpn. J. Cancer Res.*, **90**, 614–621.
22. Setiawan,V.W., Zhang,Z.F., Yu,G.P., Lu,Q.Y., Li,Y.L., Lu,M.L., Wang,M.R., Guo,C.H., Yu,S.Z., Kurtz,R.C. and Hsieh,C.C. (2001) Protective effect of green tea on the risks of chronic gastritis and stomach cancer. *Int. J. Cancer*, **92**, 600–604.
23. Nagano,J., Kono,S., Preston,D.L. and Mabuchi,K. (2001) A prospective study of green tea consumption and cancer incidence, Hiroshima and Nagasaki (Japan). *Cancer Causes Control*, **12**, 501–508.
24. Tsubono,Y., Nishino,Y., Komatsu,S., Hsieh,C.C., Kanemura,S., Tsuji,I., Nakatsuka,H., Fukao,A., Satoh,H. and Hisamichi,S. (2001) Green tea and the risk of gastric cancer in Japan. *N. Engl. J. Med.*, **344**, 632–636.
25. Inoue,M., Tajima,K., Hirose,K., Hamajima,N., Takezaki,T., Kuroishi,T. and Tominaga,S. (1998) Tea and coffee consumption and the risk of digestive tract cancers: data from a comparative case-referent study in Japan. *Cancer Causes Control*, **9**, 209–216.
26. Galanis,D.J., Kolonel,L.N., Lee,J. and Nomura,A. (1998) Intakes of selected foods and beverages and the incidence of gastric cancer among the Japanese residents of Hawaii: a prospective study. *Int. J. Epidemiol.*, **27**, 173–180.
27. Ji,B.T., Chow,W.H., Yang,G., McLaughlin,J.K., Gao,R.N., Zheng,W., Shu,X.O., Jin,F., Fraumeni,J.F.,Jr. and Gao,Y.T. (1996) The influence of cigarette smoking, alcohol and green tea consumption on the risk of carcinoma of the cardia and distal stomach in Shanghai, China. *Cancer*, **77**, 2449–2457.
28. Yu,G.P. and Hsieh,C.C. (1991) Risk factors for stomach cancer: a population-based case–control study in Shanghai. *Cancer Causes Control*, **2**, 169–174.
29. Lee,H.H., Wu,H.Y., Chuang,Y.C., Chang,A.S., Chao,H.H., Chen,K.Y., Chen,H.K., Lai,G.M., Huang,H.H. and Chen,C.J. (1990) Epidemiologic characteristics and multiple risk factors of stomach cancer in Taiwan. *Anticancer Res.*, **10**, 875–881.
30. Kono,S., Ikeda,M., Tokudome,S. and Kuratsune,M. (1988) A case–control study of gastric cancer and diet in northern Kyushu, Japan. *Jpn. J. Cancer Res.*, **79**, 1067–1074.
31. Goldbohm,R.A., Hertog,M.G., Brants,H.A., van Poppel,G. and van den Brandt,P.A. (1996) Consumption of black tea and cancer risk: a prospective cohort study. *J. Natl Cancer Inst.*, **88**, 93–100.
32. Hansson,L.E., Nyren,O., Bergstrom,R., Wolk,A., Lindgren,A., Baron,J. and Adami,H.O. (1993) Diet and risk of gastric cancer. A population-based case–control study in Sweden. *Int. J. Cancer*, **55**, 181–189.
33. Heilbrun,L.K., Nomura,A. and Stemmermann,G.N. (1986) Black tea consumption and cancer risk: a prospective study. *Br. J. Cancer*, **54**, 677–683.
34. Kinlen,L.J., Willows,A.N., Goldblatt,P. and Yudkin,J. (1988) Tea consumption and cancer. *Br. J. Cancer*, **58**, 397–401.
35. Zheng,W., Doyle,T.J., Kushi,L.H., Sellers,T.A., Hong,C.P. and Folsom,A.R. (1996) Tea consumption and cancer incidence in a prospective cohort study of postmenopausal women. *Am. J. Epidemiol.*, **144**, 175–182.
36. Arts,I.C., van de Putte,B. and Hollman,P.C. (2000) Catechin contents of foods commonly consumed in The Netherlands. 1. Fruits, vegetables, staple foods and processed foods. *J. Agric. Food Chem.*, **48**, 1746–1751.
37. Arts,I.C., van De Putte,B. and Hollman,P.C. (2000) Catechin contents of foods commonly consumed in The Netherlands. 2. Tea, wine, fruit juices and chocolate milk. *J. Agric. Food Chem.*, **48**, 1752–1757.
38. Wiseman,S.A., Balentine,D.A. and Frei,B. (1997) Antioxidants in tea. *Crit. Rev. Food Sci. Nutr.*, **37**, 705–718.
39. Ito,Y., Shimizu,H., Yoshimura,T., Ross,R.K., Kabuto,M., Takatsuka,N., Tokui,N., Suzuki,K. and Shinohara,R. (1999) Serum concentrations of carotenoids, alpha-tocopherol, fatty acids and lipid peroxides among Japanese in Japan and Japanese and Caucasians in the US. *Int. J. Vitam. Nutr. Res.*, **69**, 385–395.
40. Stich,H.F., Dunn,B.P., Pignatelli,B., Ohshima,H. and Bartsch,H. (1984) Dietary phenolics and betel nut extracts as modifiers of N-nitrosation in rat and man. In O'Neill,I.K., Von Borstel,R.C., Miller,C.T., Long,J. and Bartsch,H. (eds) *N-Nitroso Compounds*. IARC Scientific Publication, No. 57. 213–222.
41. Stich,H.F. (1992) Teas and tea components as inhibitors of carcinogen formation in model systems and man. *Prev. Med*., **21**, 377–384.
42. Nomura,A. (1996) Stomach cancer. In Schottenfeld,D. and Fraumeni,J.F. (eds) *Cancer Epidemiology and Prevention*, 2nd edn. Oxford University Press, New York, pp. 707–724.
43. Munoz,N. and Day,N.E. (1996) Esophageal cancer. In Schottenfeld,D. and Fraumeni,J.F. (eds) *Cancer Epidemiology and Prevention*, 2nd edn. Oxford University Press, New York, pp. 681–706.

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