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Original Contribution

Urinary Biomarkers of Catechins and Risk of Hepatocellular Carcinoma in the Shanghai Cohort Study

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Dietary catechins are phytochemicals with both antioxidative and prooxidative stress properties. Green tea is a major source of catechins and may be associated with hepatocellular carcinoma (HCC) risk, but the catechin-HCC relationship has not been evaluated using a biomarker-based approach. A nested case-control study of HCC (211 cases and 1,067 matched controls) was conducted within the Shanghai Cohort Study, which enrolled 18,244 men between 1986 and 1989. Concentrations of specific catechins, including epicatechin, epigallocatechin (EGC), and 4′-*O*-methyl-epigallocatechin, were measured in urine specimens that had been collected prior to HCC diagnosis. None of the catechins measured were associated with HCC risk. In stratified analyses, there was a statistically significant trend for an association of higher urinary EGC with increased HCC risk among subjects with positive serology for hepatitis B surface antigen (*P* for trend = 0.02). This positive EGC-HCC association became stronger for hepatitis B surface antigen–positive persons who also had low serum retinol levels (for detectable levels vs. undetectable levels, odds ratio = 2.62, 95% confidence interval: 1.25, 5.51). There was no evidence supporting a protective role of catechins in the development of HCC. Instead, exposure to high levels of catechins may increase the risk of developing HCC for high-risk individuals.

catechins; flavonoids; green tea; hepatocellular carcinoma; retinol

Abbreviations: CI, confidence interval; EC, epicatechin; EGC, epigallocatechin; EGCG, epigallocatechin gallate; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; 4′-MeEGC, 4′-*O*-methyl-epigallocatechin; OR, odds ratio.

Primary liver cancer is the sixth most commonly diagnosed cancer worldwide (1). There is large variation in the incidence of liver cancer across different geographical regions of the world. In 2012, China alone accounted for an estimated 50.5% of the newly diagnosed cases of liver cancer, with an incidence rate of 22.3 per 100,000 persons (1). In contrast, the United States has a relatively low incidence (the average annual rate between 2006 and 2010 was 5.9/100,000 persons), despite 5.4% and 2.3% average annual increases between 2000 and 2007 and between 2008 and 2010, respectively (2), which has been largely attributed to the peak hepatitis C virus (HCV) prevalence experienced by the aging "baby boomer" cohort (born between 1946 and 1964) (3). Most cases of hepatocellular carcinoma (HCC) develop from liver cirrhosis, which is the result of decades of liver tissue injury due to chronic infection with hepatitis B virus (HBV) and/or hepatitis C virus (HCV), alcoholic liver disease, nonalcoholic steatohepatitis, and, in certain regions, consumption of aflatoxin-contaminated foods (4, 5). The primary means of preventing the development of HCC include the reduction of aflatoxin levels in food, immunization against HBV, and antiviral treatment for HBV and HCV infection among patients with established disease. The substantial costs and adverse effects of antiviral therapies point to the need for more research to identify additional preventive agents that can be translated to primary prevention of HCC.

Epidemiologic evidence supports a role for various dietary components in reducing risk of HCC (6), such as higher serum

retinol levels (7, 8) and higher intake of fruits (9, 10) and green tea (11), as well as flavanols (12), a broad subclass of flavonoids that include catechins. Catechins are the major polyphenolic compounds in green tea, and they are also abundant in certain fruits, as well as in cocoa, broad beans, pecans, and hazelnuts (13). The catechin (−)-epigallocatechin gallate (EGCG) has been widely studied and is the most abundant of the catechins present in green tea (14). Other major catechins in green tea include (−)-epigallocatechin (EGC), (−)-epicatechingallate (ECG), and (−)-epicatechin (EC).

The chemical structure of these catechins is characterized by multiple hydroxyl groups on the 2 or more aromatic rings, contributing to its dual function as both antioxidant and prooxidant, as well as an antiinflammatory mediator (15, 16). As antioxidants, catechins decrease the generation of reactive oxygen species and maintain intracellular glutathionine in liver cells (17). Catechins have also demonstrated prooxidant properties by generating hydrogen peroxide, which promotes apoptosis, in cell lines (18–20) and in in-vivo xenograph models (21, 22). Their effects on the activation of redox-sensitive transcription factors in vitro (23) are probably due to prooxidant activities. The anti-/prooxidative duality of catechins necessitates research on their net effect on HCC risk in humans, partly because there are populations throughout the world in which consumption of catechins from green tea is relatively high.

Our research group has developed methods for quantifying levels of EC, EGC, and 4′-MeEGC (the methylated metabolite of EGC) in urine (24–26). These metabolites have been validated as biomarkers of green tea intake and have been used to establish catechin-cancer associations for esophageal, gastric, and colorectal cancers (27, 28). In the current study, we used the same method to quantify levels of EC, EGC, and 4′-MeEGC in urine samples collected from participants in the Shanghai Cohort Study without a history of cancer, to evaluate their associations with HCC risk using a nested case-control study design.

METHODS

Population

Details on the design of the Shanghai Cohort Study have been published previously (29, 30). Briefly, between January 1986 and September 1989, a total of 18,244 Chinese men (about 80% of eligible subjects) living in 4 geographically defined communities in the City of Shanghai, China, were enrolled. The eligibility criteria included age between 45 and 64 years and no history of cancer. The Shanghai Cohort Study has been approved by the institutional review boards of the Shanghai Cancer Institute (Shanghai, China) and the University of Pittsburgh (Pittsburgh, Pennsylvania).

Data collection

All subjects were interviewed in person at baseline to collect information on demographic characteristics, use of tobacco and alcohol, usual adult weight, height, diet, and medical history. An ever smoker was defined as someone who had smoked at least 1 cigarette per day for at least 6 months. An alcohol drinker was defined as someone who had consumed an alcoholic beverage at least once per week for 6 months or longer. One drink was defined as 360 g of beer (12.6 g of ethanol), 103 g of wine (12.3 g of ethanol), or 30 g of spirits (12.9 g of ethanol) (31). A positive history of liver cirrhosis was based on the subject's positive answer to the question "Have you been diagnosed with cirrhosis by a physician?" Data on tea consumption patterns were not collected during the baseline interview, but detailed questions on lifetime tea intake were included in follow-up questionnaires (28). The baseline interview was followed by the collection of a single-void urine sample and a 10-mL nonfasting blood sample. The urine and blood aliquots were stored at −70°C until laboratory analysis.

Case ascertainment

The cohort participants were followed annually for the identification of incident cancer and death. Annual visits with in-person interviews were made to each subject's home. Through December 31, 2001, the retention rate in the study was 81% among subjects who were still alive. Additionally, record reviews and linkage analysis with the databases in the population-based Shanghai Cancer Registry and the Shanghai Municipal Vital Statistics Office were conducted to complement the identification of incident cancer cases and deaths ascertained from annual home visits. By the end of 2001, a total of 214 incident HCC cases had been identified. Cases were diagnosed on the basis of histopathological confirmation, elevated serum α-fetoprotein levels with a consistent clinical and radiological history, or a positive computerized axial tomography scan and/or ultrasonograph with a consistent clinical history, or (for deceased cases) by death certificate only.

Control selection

The eligibility criteria for control subjects were being free of cancer and alive on the date of the index case's HCC diagnosis. Control subjects were matched to the index case on age at enrollment (±2 years), neighborhood of residence, and date of biospecimen collection (±1 month). In an initial study of urinary aflatoxin biomarkers in relation to HCC risk, we selected 10 controls per case for the first 6 cases to increase statistical power and then decided to reduce the number of controls to 5 per case for all later HCC cases in order to preserve the biospecimen and reduce the cost of biomarker measurements (30). Overall, 1,100 control subjects were chosen for 214 HCC cases.

Laboratory measurement

Urine samples were sorted into case-control sets and were tested in the same batch for all measurements. Laboratory personnel were blinded to the case-control status of the samples. High-performance liquid chromatography with electrochemical detection was used to quantify urinary concentrations of EC, EGC, and 4′-MeEGC (32, 33). The detection limits for EC, EGC, and 4′-MeEGC were 0.02 μg/mL, 0.01 μg/mL, and 0.04 μg/mL, respectively (32). Urinary creatinine concentration was determined in each sample using a modified method, as described previously (34). The within-batch coefficients of

variation for duplicate samples were 7.9% for EC, 11.7% for EGC, 11.2% for 4′-MeEGC, and 5.8% for creatinine. Hepatitis B surface antigen (HBsAg) serological status at baseline was determined for all subjects by means of a standard radioimmunoassay (AUSRIA; Abbott Laboratories, Abbott Park, Illinois) (35).

Statistical analysis

We conducted statistical analyses among 211 HCC cases and 1,067 matched controls, after excluding 3 cases with missing values (due to failed assays for urinary catechin or HBsAg status) and their matched controls ($n = 15$), as well as controls with missing data on urinary catechin levels and/or HBsAg status ($n = 18$). The concentrations of urinary catechins EC, EGC, and 4′-MeEGC were expressed as μmol/g creatinine (Cr) to take into account the varying water content in urine specimens (36). Log-transformed values for the catechin biomarkers were used for statistical testing to normalize the skewed distribution toward high values. We used analysis of covariance to assess differences in urinary catechin concentrations by self-reported tea intake among control subjects who answered the follow-up questionnaire ($n = 895$) and to compare the differences in urinary catechin levels between cases and controls. Pearson correlation coefficients were used to assess pairwise correlations among the 3 urinary catechin biomarkers. The χ^2 test and the t test were used to compare the distributions of proportions and means between cases and controls for selected factors, respectively.

We used standard statistical methods to analyze data for matched case-control sets (37). Conditional logistic regression models were used to calculate odds ratios and their corresponding 95% confidence intervals and P values. Comparable results were obtained in statistical analyses using unmatched case-control sets (Appendix Table 1). The referent group for each catechin consisted of all subjects with undetectable values, while the positive subjects were divided into groups according to catechin tertiles among controls.

Our goal was to estimate the least biased odds ratios for the associations between urinary catechin levels and HCC risk. We used the following approach to assess covariates for potential confounding. First, among control subjects, we compared the geometric mean values for urinary catechins in relation to selected covariates, including body mass index (weight (kg)/height (m)^2), education, smoking, alcohol drinking, HBsAg status, and history of physician-diagnosed liver cirrhosis. Second, we used conditional logistic regression methods to assess the associations between potential confounders and risk of HCC in univariate models. If a covariate was associated with urinary catechin levels (e.g., $P < 0.10$) and HCC (e.g., an odds ratio with $P < 0.10$) or if it had been previously reported to be associated with HCC risk in the study population (8, 38), we included the covariate in all adjusted models. Given the extremely low prevalence of anti-HCV positivity in our previous study (e.g., 1.3% in 76 HCC cases and 0.2% in 405 matched control subjects) (8), serological status was not determined for HCC cases that were identified later or for their matched controls, and thus was not included in the multivariable regression models. The following covariates satisfied the above criteria as potential confounders and were included in the conditional logistic regression models: smoking (never smoker, <20 cigarettes/day, or ≥20 cigarettes/day), alcohol drinking (nondrinker, <4 drinks/day, or ≥4 drinks/day), HBsAg status (negative, positive), and self-reported physician-diagnosed liver cirrhosis (no, yes).

To assess the potential modifying effect of selected risk factors on the association between catechins and HCC risk, we performed analyses stratified by smoking status and alcohol drinking, as well as by HBsAg serological status and serum retinol levels, since both have been previously determined to be important modifiers of catechin-cancer associations (8, 27). To

Table 1. Distributions of Demographic and Lifestyle Characteristics Among Hepatocellular Carcinoma Patients and Control Subjects, Shanghai Cohort Study, 1986–2001

Demographic or Lifestyle Factor	Cases ($n = 211$)		Controls ($n = 1,067$)		P Value[a]
	No.	%	No.	%	
Age at interview, years[b]	57.1 (5.0)		57.2 (5.0)		0.85
Body mass index[b,c]	22.0 (3.2)		22.2 (2.9)		0.29
Highest level of education					
No formal schooling or primary school	73	34.6	338	31.7	
Secondary school or above	138	65.4	729	68.3	0.41
Cigarette smoking status					
Never smoker	72	34.1	469	44.0	
Former smoker	22	10.4	70	6.5	
Current smoker	117	55.5	528	49.5	0.01
No. of cigarettes/day					
Never smoker	72	34.1	469	44.0	
<20	66	31.3	287	26.9	
≥20	73	34.6	311	29.1	0.03
No. of years of smoking					
Never smoker	72	34.1	469	44.0	
<30	45	21.3	230	21.5	
≥30	94	44.6	368	34.5	0.01
Alcohol intake, drinks/day					
Nondrinker	125	59.2	606	56.8	
<4	66	31.3	387	36.3	
≥4	20	9.5	74	6.9	0.23
Self-reported history of physician-diagnosed liver cirrhosis					
No	186	88.1	1,055	98.9	
Yes	25	11.9	12	1.1	<0.001
Hepatitis B surface antigen serological status					
Negative	81	38.4	964	90.4	
Positive	130	61.6	103	9.6	<0.001

[a] Two-sided P values were based on t tests for continuous variables and χ^2 tests for categorical variables.
[b] Expressed as mean (standard deviation).
[c] Weight (kg)/height (m)^2.

maximize the number of subjects in the analysis, we broke originally matched case-control sets and used unconditional logistic regression models including all matching factors to calculate odds ratios and their corresponding 95% confidence intervals and *P* values. To assess the potential latency of catechin-HCC risk associations, we performed conditional logistic regression according to different intervals of time from specimen collection to cancer diagnosis.

All statistical tests were 2-sided, and a *P* value less than 0.05 was considered statistically significant. All statistical tests were performed using the SAS software package, version 9.3 (SAS Institute, Inc., Cary, North Carolina).

RESULTS

The mean number of years from the date of urine sample collection to HCC case diagnosis was 6.9 (standard deviation, 3.8; range, 0.1–15.8 years). There were higher prevalences of smoking, history of physician-diagnosed liver cirrhosis, and positive HBsAg status in cases than in controls (Table 1). Among smokers, HCC patients had higher levels of smoking intensity and duration. Cases and controls did not differ by mean body mass index, educational level, or frequency of alcohol consumption.

We had previously established that tea drinkers in the Shanghai Cohort Study had a 2-fold increase in urinary EC levels and a 4-fold increase in both urinary EGC and 4′-MeEGC levels compared with non–tea drinkers (all *P* values < 0.001) (28). Similar relationships were demonstrated among control subjects in the present study (Table 2). Among control subjects, urinary EC, EGC, and 4′-MeEGC levels were correlated with each other (ρ = 0.50–0.64; all *P*'s < 0.001).

There was no association between increasing levels of urinary EC, EGC, and 4′-MeEGC and HCC risk (Table 3). Compared with control subjects, HCC patients had similar levels of urinary catechins and creatinine after adjustment for smoking, alcohol drinking, and HBsAg status (all *P*'s > 0.6). The geometric mean values for cases and controls, respectively, were 1.80 μmol/g Cr (95% confidence interval (CI): 1.34, 2.36) and 1.78 μmol/g Cr (95% CI: 1.32, 2.34) for EC, 5.26 μmol/g Cr (95% CI: 3.86, 7.06) and 5.00 μmol/g Cr (95% CI: 3.64, 6.78) for EGC, 15.92 μmol/g Cr (95% CI: 11.64, 21.66) and 15.18 μmol/g Cr (95% CI: 11.02, 20.80) for 4′-MeEGC, and 0.094 g/dL (95% CI: 0.083, 0.106) and 0.096 g/dL (95% CI: 0.085, 0.109) for creatinine.

Among men who were positive for HBsAg, there was a statistically significant trend of increasing HCC risk with increasing EGC level, resulting in a 2.4-fold increased risk when comparing the highest tertile with undetectable levels (Table 3). The EGC-HCC risk association was essentially null among HBsAg-negative subjects (*P* for interaction = 0.01). There were no statistically significant associations between increasing urinary catechin levels and HCC risk among subgroups defined by HBsAg status, smoking, or alcohol drinking (data not shown), with 1 exception: Among never smokers, there was an inverse association for detectable EC versus undetectable EC (odds ratio (OR) = 0.49, 95% CI: 0.27, 0.90). In analyses stratified by time interval between specimen collection and HCC diagnosis, there was no association between levels of urinary catechins and HCC risk, with 1 exception: For detectable EGC versus undetectable EGC, there was a positive association among persons with the shortest time interval between specimen collection and cancer diagnosis (<5 years: OR = 3.47, 95% CI: 1.21, 9.98; 5–<10 years: OR = 0.85, 95% CI: 0.41, 1.76; ≥10 years: OR = 0.74, 95% CI: 0.30, 1.86).

Table 2. Geometric Mean Values for Urinary Concentrations of Catechins and Their Metabolites Among Control Subjects, by Self-Reported Green Tea Intake, Shanghai Cohort Study, 1986–2001

Dry Green Tea Leaf Consumption, g/day	No. of Controls[a]	Geometric Mean, μmol/g Cr[b]		
		EC[c]	EGC[c]	4′-MeEGC[c]
0	446	1.44	3.68	11.74
<5	92	1.85	5.06	17.83
5–<10	244	2.63	7.62	21.96
≥10	113	2.80	10.53	30.90

Abbreviations: Cr, creatinine; EC, epicatechin; EGC, epigallocatechin; 4′-MeEGC, 4′-*O*-methyl-epigallocatechin.

[a] Controls with an unknown tea drinking history (*n* = 172) were excluded from the present analysis.

[b] Geometric means and *P* values (2-sided) were calculated using analysis of variance regression models.

[c] *P* for trend < 0.0001.

In Table 4, we present results for the joint associations between urinary catechins and serum retinol levels in HCC risk according to HBsAg status. Among HBsAg-positive men with a serum retinol level below the median, detectable EGC levels (versus undetectable levels) were associated with a statistically significant 2.6-fold increase in risk of HCC. This pattern of association was not observed for EC or 4′-MeEGC.

DISCUSSION

To our knowledge, the present study was the first to prospectively examine the relationship between prediagnostic urinary catechin levels and HCC risk. Using information from 211 HCC cases and 1,067 matched controls within the Shanghai Cohort Study, we found no association between levels of EC, EGC, or 4′-MeEGC and HCC risk. We did detect associations in certain subgroups, however. When data were analyzed by HBsAg serological status, a statistically significant trend in HCC risk was observed for increasing EGC levels among chronic carriers of HBV (i.e., HBsAg-positive persons). This positive association between urinary EGC and HCC risk became stronger in those with lower serum retinol levels. EGC level was also associated with risk of HCC diagnosed within the first 5 years after urine sample collection, suggesting an acute effect of catechins on HCC risk among persons with underlying disease.

Overall, our findings do not support our a priori hypothesis that higher urinary catechin levels would be associated with reduced risk of HCC. The hypothesis was based on experimental evidence that catechins, such as EGCG and EGC, have strong antioxidative properties as radical scavengers and have demonstrated inhibitory effects on carcinogenesis and cancer invasion (19, 39). In addition to their antioxidative properties, catechins exhibit chemopreventive effects as prooxidants,

Table 3. Associations Between Urinary Catechin Concentrations and Risk of Hepatocellular Carcinoma, by Hepatitis B Surface Antigen Serological Status, Shanghai Cohort Study, 1986–2001

Catechin Category	Range, μmol/g Cr	All Participants				HBsAg-Negative Participants				HBsAg-Positive Participants			
		No. of Cases (*n* = 211)	No. of Controls (*n* = 1,067)	OR[a]	95% CI	No. of Cases (*n* = 81)	No. of Controls (*n* = 964)	OR[b]	95% CI	No. of Cases (*n* = 130)	No. of Controls (*n* = 103)	OR[b]	95% CI
Epicatechin													
Undetectable	0	71	324	1.00	Referent	25	297	1.00	Referent	46	27	1.00	Referent
First tertile	>0–1.95	42	248	0.66	0.38, 1.14	16	217	0.81	0.41, 1.59	26	31	0.45	0.21, 0.96
Second tertile	1.96–5.02	45	248	0.82	0.47, 1.43	17	223	0.91	0.47, 1.75	28	25	0.56	0.26, 1.21
Third tertile	>5.02	53	247	0.98	0.57, 1.68	23	227	1.07	0.58, 1.98	30	20	0.82	0.35, 1.89
P for trend				0.97				0.80				0.47	
Detectable	>0	140	743	0.81	0.52, 1.24	56	667	0.93	0.56, 1.55	84	76	0.57	0.31, 1.05
Epigallocatechin													
Undetectable	0	42	237	1.00	Referent	18	206	1.00	Referent	24	31	1.00	Referent
First tertile	>0–5.00	51	277	1.11	0.61, 2.01	19	246	0.82	0.41, 1.64	32	31	1.44	0.65, 3.17
Second tertile	5.01–17.14	63	276	1.24	0.69, 2.20	26	254	1.15	0.60, 2.18	37	22	2.17	0.96, 4.90
Third tertile	>17.14	55	277	1.11	0.61, 2.00	18	258	0.76	0.38, 1.52	37	19	2.44	1.04, 5.71
P for trend				0.70				0.68				0.02	
Detectable	>0	169	830	1.15	0.70, 1.89	63	758	0.91	0.52, 1.59	106	72	1.92	0.98, 3.74
4′-*O*-Methyl-epigallocatechin													
Undetectable	0	35	182	1.00	Referent	15	163	1.00	Referent	20	19	1.00	Referent
First tertile	>0–16.43	47	295	0.84	0.45, 1.57	20	266	1.01	0.49, 2.08	27	29	0.80	0.32, 1.95
Second tertile	16.44–48.01	61	296	0.94	0.51, 1.70	21	267	0.94	0.46, 1.94	40	29	1.75	0.75, 4.10
Third tertile	>48.01	68	294	1.15	0.63, 2.09	25	268	1.13	0.56, 2.27	43	26	1.80	0.74, 4.41
P for trend				0.49				0.76				0.06	
Detectable	>0	176	885	0.97	0.57, 1.64	66	801	1.03	0.56, 1.89	110	84	1.39	0.66, 2.92

Abbreviations: CI, confidence interval; Cr, creatinine; HBsAg, hepatitis B surface antigen; OR, odds ratio.

[a] Conditional logistic regression models included the following covariates: HBsAg status, self-reported history of physician-diagnosed liver cirrhosis, smoking (nonsmoker, <20 cigarettes/day, or ≥20 cigarettes/day), and alcohol intake (nondrinker, <4 drinks/day, or ≥4 drinks/day).

[b] Unconditional logistic regression models included all of the covariates listed in footnote "a" above, as well as matching factors (i.e., age, year of sample collection, and neighborhood of residence at enrollment).

Table 4. Joint Effects of Urinary Catechin and Serum Retinol Concentrations on Risk of Hepatocellular Carcinoma, by Hepatitis B Surface Antigen Serological Status, Shanghai Cohort Study, 1986–2001

Retinol Level and Catechin Category	HBsAg-Negative Participants				HBsAg-Positive Participants			
	No. of Cases ($n = 81$)	No. of Controls ($n = 963$)	OR[a]	95% CI	No. of Cases ($n = 130$)	No. of Controls ($n = 102$)	OR[a]	95% CI
Retinol <45.8 μg/dL								
Epicatechin								
Undetectable	19	150	1.00	Referent	42	17	1.00	Referent
Detectable	40	320	0.90	0.50, 1.64	74	48	0.55	0.27, 1.12
Retinol ≥45.8 μg/dL								
Epicatechin								
Undetectable	6	146	0.33	0.13, 0.87	4	10	0.17	0.04, 0.66
Detectable	16	347	0.36	0.18, 0.74	10	27	0.12	0.05, 0.34
Retinol <45.8 μg/dL								
Epigallocatechin								
Undetectable	14	108	1.00	Referent	21	24	1.00	Referent
Detectable	45	362	0.86	0.45, 1.66	95	41	2.62	1.25, 5.51
Retinol ≥45.8 μg/dL								
Epigallocatechin								
Undetectable	4	98	0.30	0.09, 0.95	3	7	0.48	0.10, 2.39
Detectable	18	395	0.35	0.17, 0.74	11	30	0.40	0.15, 1.09
Retinol <45.8 μg/dL								
4′-*O*-Methyl-epigallocatechin								
Undetectable	10	77	1.00	Referent	19	13	1.00	Referent
Detectable	49	393	1.05	0.49, 2.23	97	52	1.39	0.59, 3.27
Retinol ≥45.8 μg/dL								
4′-*O*-Methyl-epigallocatechin								
Undetectable	5	86	0.43	0.14, 1.36	1	6	0.10	0.01, 1.05
Detectable	17	407	0.38	0.17, 0.89	13	31	0.30	0.11, 0.85

Abbreviations: CI, confidence interval; HBsAg, hepatitis B surface antigen; OR, odds ratio.

[a] Unconditional logistic regression models included the following covariates: age, year of sample collection, neighborhood of residence at enrollment, self-reported history of physician-diagnosed liver cirrhosis, smoking (nonsmoker, <20 cigarettes/day, or ≥20 cigarettes/day), and alcohol intake (nondrinker, <4 drinks/day, or ≥4 drinks/day).

generating hydrogen peroxide, inducing apoptosis, and ultimately inhibiting tumor proliferation (19–21, 40). In mice, hepatotoxic effects of high-dose EGCG were associated with increased markers of oxidative stress, including hepatic lipid peroxidation and plasma 8-isoprostane (41). These prooxidative properties may also be partly responsible for the documented hepatic toxicity associated with consumption of green tea, even at frequencies as low as 6 cups per day (16). Hepatotoxicity resulting in an acute hepatitis-like syndrome has also been documented in users of green tea extract supplements (42, 43). Liver biopsy findings from such cases demonstrate patterns consistent with acute hepatitis, such as necrosis, inflammation, and the presence of eosinophils (44). However, the doses of EGCG that caused toxicity in humans correspond to approximately 10.5–32 cups of green tea per day, and thus would not suggest a high risk of hepatotoxicity in healthy persons consuming 2–3 cups per day. It is biologically plausible, however, that the risk of hepatotoxicity induced by catechin intake may be enhanced among persons with chronic hepatitis B and/or cirrhosis.

Chronic infection with HBV and/or HCV is the strongest risk factor for HCC (45). The persistent inflammation associated with chronic hepatitis produces substantial amounts of inflammatory cytokines and reactive oxygen species, which may further enhance liver injury and inflict DNA damage that can eventually lead to carcinogenesis (46, 47). Retinoids can protect against liver injury induced by chronic inflammation that is consistent with hepatitis infection (48, 49). Retinol has also demonstrated its protection against reactive oxygen species–induced DNA damage in hepatoma cells (50). Thus, our observation of a statistically significant positive association between urinary EGC levels and HCC risk among HBsAg-positive persons with low serum retinol levels can be explained by a mechanism of prooxidative action by EGCG and other catechins. This is similar to the observation that higher doses of EGCG exacerbated the inflammatory conditions

induced by dextran sulfate sodium in a mouse colon model (51).

Using a similar nested case-control study design and biomarker approach, we previously reported statistically significant inverse associations between urinary levels of EGC (but not EC) and risk of esophageal, gastric, or colon cancer (27, 28). EGC is a more specific marker of green tea intake than is EC, given that the non–green tea dietary sources of EGC are relatively minor compared with EC (52). Given the same laboratory using the same quantification method, the present analysis, which had an identical design within the same prospective cohort study, strongly supports an overall lack of association between these measured catechins and risk of HCC.

Overall, there is some support for an inverse relationship between green tea intake and liver cancer risk. In a meta-analysis of 13 epidemiologic studies (including 7 prospective cohort studies), daily green tea consumption was associated with a statistically nonsignificant reduced risk of HCC (OR = 0.77, 95% CI: 0.57, 1.03; $P = 0.08$) (11). All studies but 1 were conducted in Chinese or Japanese populations, where green tea is the primary type of tea consumed. A more recent study among Chinese women in Shanghai did not find a statistically significant association between regular green tea consumption (i.e., ≥3 times per week for >6 months) and liver cancer risk (hazard ratio = 0.89, 95% CI: 0.58, 1.38) (53). Only 1 of the previous prospective cohort studies reported on the association between tea consumption and HCC risk among persons with chronic HBV or HCV infection: In a Japanese study, Inoue et al. (54) demonstrated a positive, statistically borderline-significant elevation of HCC risk for men with positive anti-HCV and/or HBsAg serology who consumed 5 or more cups of green tea per day relative to fewer than 3 cups per day (hazard ratio = 1.70, 95% CI: 0.85, 3.41; P for trend = 0.062). These results support our finding of a potentially adverse impact of heavy green tea intake on HCC risk among high-risk populations.

The primary strength of our study was the use of an objective biomarker of levels of urinary catechins and their metabolites to evaluate associations between individual catechin levels and HCC risk rather than relying on self-reported tea intake. The assay, with excellent within-batch correlations, produced biomarker measurements that were valid, since all samples were tested within a single batch. Another strength was our ability to adjust for potential confounding due to HBsAg serological status, smoking history, and alcohol intake, among other factors. A limitation of our study was the collection of a spot urine specimen. It may be preferable to collect 24-hour urine specimens, but there were obvious feasibility concerns in a large study such as this one. Chinese people typically drink green tea after a meal as a digestive aid. The mean amounts of time between the last meal and urine collection were similar (about 3 hours) between cases and controls in the present study ($P = 0.7$). It is possible that given the relatively short elimination half-lives of catechins (2–3 hours) (24), infrequent green tea drinkers may have been misclassified as having lower catechin levels, regardless of case status. Thus, the failure of the study to detect statistically significant associations could have been due to these limitations, especially if the true association between catechins and cancer risk is moderate. Another limitation was that our subgroup finding by HBsAg status and retinol level could have been due to chance, given the small number of subjects (e.g., $n < 10$) in several cells and/or the multiple comparisons performed. It is also possible that our finding of a strong positive association between EGC and HCC among persons with the shortest time interval between specimen collection and cancer diagnosis was due in part to greater tea intake among persons with underlying disease.

In summary, the present study did not show an overall inverse association between catechins found in green tea and HCC risk. Instead, high consumption of green tea may pose some unanticipated risk to people at high risk of HCC, such as patients with chronic hepatitis or liver cirrhosis. Although there is abundant evidence in support of many beneficial health effects of green tea, green tea or its catechins may not be a viable chemopreventive agent against the development of HCC in humans.

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Appendix Table 1. Unconditional Odds Ratios[a] for Hepatocellular Carcinoma in Relation to Urinary Concentrations of Catechins and Their Metabolites, Shanghai Cohort Study, 1986–2001

Catechin	Tertile of Urinary Catechin Concentration								P_{trend}
	Undetectable		First Tertile		Second Tertile		Third Tertile		
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	
Epicatechin	1.00	Referent	0.64	0.39, 1.06	0.78	0.48, 1.27	0.96	0.59, 1.54	0.850
Epigallocatechin	1.00	Referent	1.13	0.67, 1.90	1.54	0.92, 2.58	1.23	0.72, 2.09	0.280
4′-*O*-Methyl-epigallocatechin	1.00	Referent	0.93	0.52, 1.64	1.18	0.69, 2.03	1.32	0.77, 2.28	0.175

Abbreviations: CI: confidence interval; OR, odds ratio.

[a] Unconditional logistic regression models included the following covariates: age, year of sample collection, neighborhood of residence at enrollment, hepatitis B surface antigen status, self-reported history of physician-diagnosed liver cirrhosis, smoking (nonsmoker, <20 cigarettes/day, or ≥20 cigarettes/day), and alcohol intake (non-drinker, <4 drinks/day, or ≥4 drinks/day).