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Green Tea Consumption Is Inversely Associated with the Incidence of Influenza Infection among Schoolchildren in a Tea Plantation Area of Japan[1–3]

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Abstract

Green tea is known to contain antiviral components that prevent influenza infection. A limited number of adult clinical studies have been undertaken, but there is a paucity of clinical evidence concerning children. We conducted an observational study to determine the association between green tea consumption and the incidence of influenza infection among schoolchildren. Anonymous questionnaire surveys were undertaken twice during the influenza season from November 2008 to February 2009 (endemic seasonal type A influenza infection); each survey was conducted for 2663 pupils across all elementary schools in Kikugawa City (a tea plantation area), Japan. Each questionnaire was completed and submitted by 2050 pupils (response rate, 77.0%; age range, 6–13 y). The adjusted OR associated with the consumption of green tea for ≥6 d/wk compared with <3 d/wk was 0.60 [(95% CI = 0.39–0.92); P = 0.02] in cases of influenza confirmed by the antigen test. Meanwhile, the adjusted OR inversely associated with the consumption of 1 cup/d to <3 cups/d (1 cup = 200 mL) and 3–5 cups/d compared with <1 cup/d were 0.62 [(95% CI = 0.41–0.95); P = 0.03] and 0.54 [(95% CI = 0.30–0.94); P = 0.03], respectively. However, there was no significant association with the consumption of >5 cups/d. Our findings thus suggest that the consumption of 1–5 cups/d of green tea may prevent influenza infection in children.
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Introduction

Influenza infection is prevalent worldwide during endemic or epidemic seasons; it spreads easily among elementary schoolchildren and from them to the rest of the community. The disease burden of influenza infection is considerable, leading to a disruption of school life because of the absence of pupils or the closure of schools (1–3). Moreover, serious complications such as pneumonia or encephalitis occasionally occur (4,5). Thus, the prevention of influenza infection is very important.

The recommended preventive measures for influenza fall into 2 categories. The first includes pharmaceutical measures, such as influenza vaccination (6–8) and neuraminidase inhibitors [oseltamivir (Tamiflu) and zanamivir (Relenza)] for prophylaxis (9,10). The second category includes nonpharmaceutical measures, such as masks, hand hygiene, gargling, and avoiding crowds (11–14).

Green tea catechins are reported to have various physiological activities, including antioxidative, anticancer, hypolipidemic, hypoglycemic, hypotensive, antibacterial, and antiviral effects (15–19). Moreover, in vitro studies have revealed that green tea catechins inhibit viral infectivity and proliferation by blocking adsorption, hemagglutination, virus assembly, or maturation cleavage (20–22). Among the nonpharmaceutical prophylactic measures against influenza infection, however, only a few clinical studies have investigated the antiviral preventive effects of green tea catechins (23,24). A recent study reported that the consumption of a specific formulation of tea extracts enhances systemic immunity ($\gamma\delta$ T-cell function) and prevents the occurrence of cold and flu symptoms in healthy adults (25). Furthermore, a randomized controlled study reported that taking green tea components reduced the incidence of influenza infection among healthcare workers for the elderly (26). However, there is little evidence indicating that the consumption of green tea can prevent influenza infection in children.

Given this background, we conducted an observational study to determine the association between the consumption of green

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[3] Supplemental Figure 1 is available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at jn.nutrition.org.
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tea and the incidence of influenza infection among elementary schoolchildren in a tea plantation area of Japan.

Methods

Study design. During the seasonal influenza period from November 2008 to February 2009, an anonymous questionnaire survey was undertaken to detect the incidence of influenza infection and the preventive measures used; the survey was conducted for 2663 pupils of all the elementary schools (9 schools) in Kikugawa City (a tea plantation area), Japan. The participants initially answered a set of questions regarding their state of health (from April–October 2008) and then completed the first (from November–December 2008) and second (from January–February 2009) questionnaires. A serial registration number was printed on each questionnaire. The questionnaire set was distributed among the pupils in December. The response to the set of questions regarding their state of health was collected prior to completion of the first questionnaire at the beginning of January 2009; the second questionnaire was collected at the beginning of March 2009. In the first sentence of each questionnaire, we requested parents to read the questionnaire with their child and to help answer the questions. The study protocol was approved by the Ethics Committee of the University of Shizuoka.

Contents of the questionnaires. In the set of questions regarding the state of health before this survey, the number of days of absence from school because of poor health from April–October 2008 was asked. The following items were then surveyed in the first and second questionnaires: the incidence of influenza infection (including influenza-like illness), symptoms, and recovery states from influenza infection; the findings of the influenza antigen test; the number of days of absence from school, or the number of days of hospitalization because of influenza infection; the risk of influenza infection by household transmission; influenza vaccination status; the frequency and quantity of green tea consumption; the frequency of preventive measures such as hand hygiene, facemasks, and gargling; sufficient nourishment; sufficient sleep; thermal insulation; humidification; ventilation; and the avoidance of crowds (except at school).

To protect the privacy of the children surveyed, we did not collect information regarding underlying or chronic diseases; instead, we queried the number of days of absence from school because of poor health before this survey (April–October 2008), as an indicator of the child's general state of health prior to the study period.

In our study, a child affected by influenza was defined as a child diagnosed with influenza or suspected to have influenza by medical doctors on the basis of clinical signs (such as fever, cough, arthralgia, runny nose, and headache) and antigen test results. The antigen test was performed by an immunochromatographic assay using a nasopharyngeal swab specimen with ~85% sensitivity and 100% specificity for the influenza virus type A and B antigens (23,24).

The risk of influenza infection by household transmission was evaluated using the following criteria. If one or more members of the child's household were suffering from influenza, the child was considered to be at risk of influenza infection by household transmission. In other words, if a child infected with influenza during the study period was not the first influenza patient in his/her household, the risk of infection by household transmission was considered present. However, if a child infected with influenza during the study period was the first patient in the household, the risk of household transmission was considered absent.

To confirm and avoid mixing the data for the preventive measures used before pupils were infected with influenza, those pupils infected with influenza during the survey period were required to detail their circumstances before and after infection.

Statistical analysis. Statistical analyses were performed for the follow-up data from the first and second questionnaires. For the frequency (d/wk) and quantity (cups/d) of green tea consumption and the frequency of each preventive measure, we calculated the mean of the data from the first and second questionnaires for each serial registration number. For the frequency of green tea consumption and each preventive measure in children infected with influenza, we analyzed only the data acquired before infection (i.e. the data of the measures undertaken to prevent infection).

For the frequency (d/wk) of green tea consumption and each preventive measure, we further classified the data into the following 3 categories: <3 d/wk (considered as sometimes or less); 3 to <6 d/wk (often); and >6 d/wk (almost every day). We classified quantity (cups/d; 1 cup = 200 mL) of green tea consumption into the following 4 categories: <1, 1 to <3, 3–5, and >5 cups/d.

It is difficult to determine the frequency of wearing a mask, because masks tend to be worn only during the fixed period when influenza is prevalent; therefore, we classified mask usage for the prevention of influenza into 2 categories, i.e. used or not used.

We conducted logistic regression analysis to determine the association between background factors or each preventive measure and the incidence of influenza infection. Influenza infection was classified into 2 categories: clinical influenza and confirmed influenza. Clinical influenza included all children diagnosed with influenza or suspected to have influenza by medical doctors on the basis of clinical signs, regardless of an antigen test being conducted or its result (i.e. positive or negative). Confirmed influenza included only children confirmed by medical doctors to have influenza based on a positive antigen test.

Next, we conducted logistic regression analysis to determine the association between the consumption of green tea and the incidence of influenza infection. We used forced-entry methods in 2 multivariate logistic regression models to include plausible confounding factors. Model 1 was adjusted for background factors [age, gender, the number of days of absence from school because of poor health before this survey (April–October 2008), and the risk of influenza infection by household transmission], influenza vaccination status, frequency of hand hygiene, and facemask use, which are the most commonly used methods for influenza prevention. Model 2 was adjusted for all of the confounding factors of Model 1, as well as gargling, nourishment, sleep, thermal insulation, humidification, ventilation, and avoiding crowds (except at school), which are recommended for the prevention of influenza (11–14). The prophylactic use of neuraminidase inhibitors is not common in Japan and is not covered by medical insurance and therefore we did not include this as a confounding factor in our analysis.

To determine the association between the consumption of green tea and the number of days of absence from school because of influenza infection, we conducted linear regression analysis for confirmed influenza and clinical influenza. For multiple regression models, we also used forced-entry methods to include plausible confounding factors such as background factors [age, gender, the number of days of absence from school because of poor health before this survey was conducted (April–October 2008)], influenza vaccination status, and antiviral status for influenza treatment, which are known to mitigate the symptoms of influenza and facilitate recovery (6–10). We excluded from our analysis any child infected with influenza during a holiday or winter vacation who was therefore not classified as being absent from school.

All statistical analyses were performed using SPSS for Windows, version 17.0 (SPSS). $P < 0.05$ was considered significant.

Results

Incidence of influenza infection

An endemic seasonal outbreak of type A influenza was widespread in Japan throughout the study period (from November 2008 to February 2009). Of the 2663 pupils to whom the survey was administered, 2395 completed the details of their state of health before completing the questionnaire survey, 2476 completed the first questionnaire, and 2119 completed the second questionnaire. We excluded 613 pupils excluded from the final analysis either because of noncompletion of the 2 questionnaires or because of missing data, including that of the main outcome measures. Thus, 2050 pupils (response rate, 77.0%; age range, 6–13 y) were included in the final analysis (**Supplemental Fig. 1**).

The number of reported episodes of clinical influenza infection was 241 (11.8%), of which 204 (10.0%) were confirmed by

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TABLE 1 Distribution of green tea consumption by demographic and potential confounding factors and OR for the incidence of influenza infection according to confounding factors for elementary schoolchildren in Kikugawa City, Japan[1,2]

	Green tea consumption (d/wk)			Green tea consumption[3] (cups/d)				Incidence of influenza infection				
									Confirmed flu[4]		Clinical flu[5]	
	<3	3 to <6	≥6	<1	1 to <3	3–5	>5	*n*	OR	95%CI	OR	95%CI
Respondent,[6] *n (%)*	392 (20.1)	530 (27.1)	1032 (52.8)	597 (38.7)	595 (38.6)	316 (20.5)	35 (2.3)	—	—	—	—	—
Influenza infection status												
Confirmed influenza infection, *n* (%)	47 (12.0)	53 (10.0)	88 (8.5)	79 (13.2)	50 (8.4)	26 (8.2)	7 (20.0)	—	—	—	—	—
Clinical influenza infection, *n* (%)	55 (14.0)	67 (12.6)	99 (9.6)	95 (15.9)	61 (10.3)	28 (8.9)	7 (20.0)	—	—	—	—	—
Baseline characteristics												
Age								2040	0.87	0.80, 0.95	0.89	0.82, 0.96
n	390	529	1026	594	592	316	35					
Age, *y*	9.1 ± 1.7	9.4 ± 1.7	9.3 ± 1.7	9.2 ± 1.7	9.3 ± 1.7	9.5 ± 1.7	9.5 ± 1.9					
Days absent from school (Apr-Oct, 2008)[7]								1937	1.05	0.98, 1.12	1.12	1.04, 1.20
n	366	499	982	560	567	297	35					
School absence, *d*	1.0 ± 2.6	0.8 ± 1.4	0.8 ± 1.5	0.8 ± 2.2	0.8 ± 1.5	0.7 ± 1.3	1.2 ± 2.0					
Sex												
n	392	530	1032	597	595	316	35					
Male, %	55.6	46.4	47.3	52.9	46.9	43.7	42.9	991	1.00	Reference	1.00	Reference
Female, %	44.4	53.6	52.7	47.1	53.1	56.3	57.1	1059	0.78	0.58, 1.04	0.81	0.62, 1.05
Risk of influenza infection by household transmission												
n	385	516	1024	583	589	313	34					
No risk,%	84.9	84.3	85.4	83.7	82.9	87.9	91.2	1710	1.00	Reference	1.00	Reference
Risk, %	15.1	15.7	14.6	16.3	17.1	12.1	8.8	303	2.80	2.00, 3.91	2.52	1.83, 3.47
Preventive measures for influenza infection												
Influenza vaccination status												
n	376	516	1016	580	585	308	34					
Not vaccinated, %	42.6	48.3	40.6	44.5	43.1	41.6	35.3	854	1.00	Reference	1.00	Reference
Vaccinated, %	57.4	51.7	59.4	55.5	56.9	58.4	64.7	1141	1.20	0.89, 1.62	1.18	0.89, 1.56
Facemask use												
n	355	497	976	558	562	297	34					
No use, %	70.7	64.8	66.1	69.0	64.8	66.0	55.9	1268	1.00	Reference	1.00	Reference
Use, %	29.3	35.2	33.9	31.0	35.2	34.0	44.1	636	0.94	0.69, 1.30	0.96	0.71, 1.29
Hand hygiene												
n	302	433	866	488	504	256	32					
<3 d/wk, %	4.3	2.5	1.3	4.1	1.2	2.0	0.0	37	1.00	Reference	1.00	Reference
3 to < 6 d/wk, %	19.9	18.9	11.8	17.2	14.5	12.9	0.0	252	0.70	0.25, 1.97	1.00	0.36, 2.74
≥6 d/wk, %	75.8	78.5	87.0	78.7	84.3	85.2	100	1353	0.71	0.27, 1.85	0.83	0.32, 2.16
Gargling												
n	384	510	1020	586	588	312	33					
<3 *d/wk, %*	26.6	24.3	17.2	24.1	15.6	16.7	21.2	434	1.00	Reference	1.00	Reference
3 to < 6 *d/wk, %*	29.9	33.3	25.7	28.8	28.7	26.6	12.1	573	0.98	0.65, 1.48	1.13	0.77, 1.66
≥6 *d/wk, %*	43.5	42.4	57.2	47.1	55.6	56.7	66.7	993	0.96	0.66, 1.40	0.99	0.69, 1.41

(Continued)

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TABLE 1 *Continued*

	Green tea consumption (d/wk)			Green tea consumption[3] (cups/d)				Incidence of influenza infection				
									Confirmed flu[4]		Clinical flu[5]	
	<3	3 to <6	≥6	<1	1 to <3	3–5	>5	*n*	OR	95%CI	OR	95%CI
Sufficient nourishment												
n	357	499	978	559	567	297	34					
<3 *d/wk*, %	9.5	7.2	5.6	7.3	6.9	5.1	8.8	135	1.00	Reference	1.00	Reference
3 to < 6 *d/wk*, %	18.8	17.6	17.7	19.0	19.2	16.2	17.6	340	0.96	0.52, 1.79	0.84	0.47, 1.50
≥6 *d/wk*, %	71.7	75.2	76.7	73.7	73.9	78.8	73.5	1436	0.77	0.44, 1.33	0.78	0.47, 1.30
Sufficient sleep												
n	361	501	987	563	571	300	34					
<3 *d/wk*, %	10.5	8.2	7.4	9.6	6.0	8.3	8.8	159	1.00	Reference	1.00	Reference
3 to < 6 *d/wk*, %	26.6	33.7	26.8	30.2	30.5	26.7	26.5	546	1.10	0.62, 1.97	0.97	0.57, 1.63
≥6 *d/wk*, %	62.9	58.1	65.8	60.2	63.6	65.0	64.7	1222	0.93	0.54, 1.61	0.80	0.49, 1.30
Thermal insulation												
n	355	500	980	555	570	299	33					
<3 *d/wk*, %	19.4	13.4	13.1	17.3	13.0	9.0	3.0	279	1.00	Reference	1.00	Reference
3 to < 6 *d/wk*, %	16.9	17.6	12.7	15.5	15.6	11.0	15.2	282	1.57	0.86, 2.87	1.63	0.94, 2.83
≥6 *d/wk*, %	63.7	69.0	74.3	67.2	71.4	79.9	81.8	1349	1.55	0.94, 2.55	1.48	0.93, 2.33
Humidification												
n	359	496	987	559	571	301	34					
<3 *d/wk*, %	60.4	62.7	59.8	59.7	59.0	59.8	58.8	1157	1.00	Reference	1.00	Reference
3 to < 6 *d/wk*, %	14.2	12.5	10.8	15.6	11.7	11.3	5.9	230	1.48	0.95, 2.31	1.32	0.87, 2.02
≥6 *d/wk*, %	25.3	24.8	29.4	24.7	29.2	28.9	35.3	529	1.37	0.97, 1.92	1.23	0.90, 1.69
Ventilation												
n	360	498	983	561	571	299	34					
<3 *d/wk*, %	35.8	30.9	27.4	33.5	27.8	21.1	20.6	575	1.00	Reference	1.00	Reference
3 to < 6 *d/wk*, %	23.1	27.1	21.0	24.8	24.5	19.4	29.4	442	1.17	0.74, 1.83	1.20	0.80, 1.81
≥6 *d/wk*, %	41.1	42.0	51.7	41.7	47.6	59.5	50.0	899	1.58	1.09, 2.28	1.44	1.03, 2.03
Avoiding crowds[8]												
n	354	496	970	555	560	298	33					
<3 *d/wk*, %	63.0	57.9	58.9	58.7	56.8	59.7	42.4	1126	1.00	Reference	1.00	Reference
3 to < 6 *d/wk*, %	23.2	24.6	24.9	25.9	26.3	24.8	30.3	460	0.84	0.57, 1.22	0.95	0.67, 1.34
≥6 *d/wk*, %	13.8	17.5	16.2	15.3	17.0	15.4	27.3	305	0.96	0.63, 1.46	1.02	0.69, 1.51

[1] Values are mean ± SD, number, or percentage.
[2] Data were collected from November, 2008 to February, 2009.
[3] 1 cup = 200 mL.
[4] Confirmed flu = laboratory confirmed influenza infection.
[5] Clinical flu = clinically defined influenza infection.
[6] Of the 2050 pupils analyzed, 96 responses for d/wk and 507 for cup/d were missing.
[7] Absent due to illness only.
[8] Avoiding crowds, except at school.

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TABLE 2 OR (95% CI) for the incidence of influenza infection for elementary schoolchildren in Kikugawa City by level of green tea consumption (d/wk)

	Green tea consumption (d/wk)									
	<3		3 to <6				≥6			
	n	OR	*n*	OR	95% CI	*P*	*n*	OR	95% CI	*P*
Confirmed influenza										
Unadjusted	392	1.00	530	0.82	0.54, 1.24	0.34	1032	0.68	0.47, 1.00	0.05
Model 1[1]	270	1.00	391	0.63	0.39, 1.04	0.07	801	0.60	0.39, 0.92	0.02
Model 2[2]	259	1.00	373	0.62	0.37, 1.05	0.07	765	0.60	0.39, 0.95	0.03
Clinical influenza										
Unadjusted	392	1.00	530	0.89	0.60, 1.30	0.54	1032	0.65	0.46, 0.92	0.02
Model 1[1]	270	1.00	391	0.77	0.49, 1.23	0.27	801	0.62	0.41, 0.93	0.02
Model 2[2]	259	1.00	373	0.76	0.47, 1.23	0.26	765	0.63	0.41, 0.97	0.03

[1] Adjusted for potential confounding variables of baseline characteristics (listed in Table 1), influenza vaccination status, facemasks, and frequency of hand hygiene.
[2] Adjusted for all potential confounding variables of baseline characteristics and preventive measures listed in Table 1.

the antigen test (185 cases of influenza A; 18 cases of influenza B; 1 case of combined influenza A and B). No children were hospitalized during this period.

Green tea consumption and the incidence of influenza infection

Distribution and confounding factors. Of the 2050 pupils analyzed, 1954 (73.5%) answered the question regarding the frequency of green tea consumption (d/wk) and 1543 (57.9%) answered the question regarding the quantity of green tea consumption (cups/d). More than 50% of respondents drank green tea at a frequency of >6 d/wk (**Table 1**). For the quantity of green tea consumption, a large number (77.3%) of pupils drank <1 cup/d and 1 to < 3 cups/d, with approximately the same number of pupils in each group. For all categories of green tea consumption, >50% of pupils had undergone influenza vaccination.

Age was inversely associated with the incidence of confirmed and clinical influenza infection (Table 1). The number of days of absence from school because of poor health before the survey (April–October 2008) was associated with the incidence of clinical influenza infection. The risk of influenza infection by household transmission was strongly associated with the incidence of confirmed influenza [OR = 2.80 (95% CI = 2.00–3.91); $P < 0.001$] and clinical influenza infection [OR = 2.52 (95% CI = 1.83–3.47); $P < 0.001$]. However, general preventive measures, such as influenza vaccination, hand hygiene, and the use of facemasks, were not associated with the incidence of influenza infection. Paradoxically, ventilation was associated with the incidence of influenza infection.

Frequency of green tea consumption (d/wk) and influenza infection. The incidence of clinical and confirmed influenza was inversely associated with the consumption of green tea for ≥6 d/wk compared with <3 d/wk (**Table 2**). In the multivariate logistic regression model 1, the adjusted OR inversely associated with the consumption of green tea for ≥6 d/wk compared with <3 d/wk were 0.60 [(95% CI = 0.39–0.92); $P = 0.02$] and 0.62 [(95% CI = 0.41–0.93); $P = 0.02$] in cases of confirmed and clinical influenza, respectively. In model 2, the adjusted OR associated with the consumption of green tea for ≥6 d/wk compared with <3 d/wk were 0.60 [(95% CI = 0.39–0.95); $P = 0.03$] and 0.63 [(95% CI = 0.41–0.97); $P = 0.03$] in cases of confirmed and clinical influenza, respectively.

Quantity of green tea consumption (cups/d) and influenza infection. The incidence of clinical and confirmed influenza was inversely associated with the consumption of 1–5 cups/d of green

TABLE 3 OR (95% CI) for the incidence of influenza infection for elementary schoolchildren in Kikugawa City by level of green tea consumption (cup/d)

	Green tea consumption (cups/d)[1]													
	<1		1 to <3				3–5				>5			
	n	OR	*n*	OR	95% CI	*P*	*n*	OR	95% CI	*P*	*n*	OR	95% CI	*P*
Confirmed influenza														
Unadjusted	597	1.00	595	0.60	0.41, 0.87	0.008	316	0.59	0.37, 0.94	0.03	35	1.64	0.69, 3.88	0.26
Model 1[2]	436	1.00	463	0.62	0.41, 0.95	0.03	237	0.54	0.30, 0.94	0.03	31	1.42	0.51, 3.97	0.50
Model 2[3]	416	1.00	445	0.62	0.39, 0.96	0.03	231	0.50	0.28, 0.90	0.02	29	1.33	0.46, 3.85	0.60
Clinical influenza														
Unadjusted	597	1.00	595	0.60	0.43, 0.85	0.004	316	0.51	0.33, 0.80	0.003	35	1.32	0.56, 3.11	0.52
Model 1[2]	436	1.00	463	0.65	0.43, 0.97	0.03	237	0.54	0.31, 0.92	0.02	31	1.22	0.44, 3.38	0.70
Model 2[3]	416	1.00	445	0.64	0.42, 0.97	0.04	231	0.51	0.29, 0.89	0.02	29	1.17	0.40, 3.38	0.77

[1] 1 cup = 200 mL.
[2] Adjusted for potential confounding variables of baseline characteristics (listed in Table 1), influenza vaccination status, facemasks, and frequency of hand hygiene.
[3] Adjusted for all potential confounding variables of baseline characteristics and preventive measures listed in Table 1.

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TABLE 4 Distribution of green tea consumption by demographic and potential confounding factors and regression analyses of the days of absence from school following influenza infection and each confounding factor in 204 patients (confirmed influenza) and 241 patients (clinical influenza) for elementary schoolchildren in Kikugawa City, Japan[1,2]

	Green tea consumption (d/wk)			Green tea consumption[3] (cups/d)				Days of absence from school by influenza infection			
	<3	3 to <6	≥6	<1	1 to <3	3–5	>5	R^2	F	β	P
Respondent[4]											
Confirmed flu, *n*	47	53	88	79	50	26	7	—	—	—	—
Clinical flu, *n*	55	67	99	95	61	28	7	—	—	—	—
School absence days by influenza infection											
Confirmed flu, *d*	4.2 ± 1.4	3.4 ± 1.4	3.6 ± 1.5	3.9 ± 1.5	3.4 ± 1.4	3.8 ± 2.0	3.7 ± 1.5	—	—	—	—
n	47	52	87	78	50	25	7				
Clinical flu, *d*	4.4 ± 2.0	3.5 ± 1.5	3.7 ± 1.5	4.1 ± 1.8	3.6 ± 1.4	3.9 ± 2.0	3.7 ± 1.5	—	—	—	—
n	55	65	98	93	61	27	7				
Age											
Confirmed flu, *y*	9.0 ± 1.4	9.0 ± 1.4	9.0 ± 1.8	9.0 ± 1.5	9.0 ± 1.8	9.2 ± 1.8	9.0 ± 1.7	0.01	2.84	−0.12	0.09
n	47	53	87	79	49	26	7				
Clinical flu, *y*	9.0 ± 1.4	8.9 ± 1.4	9.1 ± 1.7	9.0 ± 1.5	9.1 ± 1.8	9.1 ± 1.7	9.0 ± 1.7	0.01	2.85	−0.11	0.09
n	55	67	98	95	60	28	7				
Sex											
Confirmed flu, *n*	47	53	88	79	50	26	7	<0.01	0.07		
Male, %	57.4	50.9	54.5	57.0	52.0	61.5	42.9			Reference	Reference
Female, %	42.6	49.1	45.5	43.0	48.0	38.5	57.1			−0.02	0.80
Clinical flu, *n*	55	67	99	95	61	28	7	<0.01	0.03		
Male, %	58.2	49.3	53.5	54.7	49.2	64.3	42.9			Reference	Reference
Female, %	41.8	50.7	46.5	45.3	50.8	35.7	57.1			0.01	0.86
Days absent from school (Apr-Oct, 2008)[5]											
Confirmed flu, *d*	0.8 ± 1.6	0.9 ± 1.3	0.9 ± 1.3	0.9 ± 1.5	1.0 ± 1.3	0.6 ± 1.0	0.6 ± 1.0	<0.01	1.21	0.08	0.27
n	45	51	83	75	50	24	7				
Clinical flu, *d*	1.8 ± 5.8	0.8 ± 1.3	1.0 ± 1.9	1.4 ± 4.5	1.3 ± 2.2	0.6 ± 1.0	0.6 ± 1.0	0.11	27.17 ***	0.33	< 0.001
n	52	64	94	89	61	26	7				
Influenza vaccination status											
Confirmed flu, *n*	45	51	88	76	50	26	7	0.03	6.15*		
Not vaccinated, %	48.9	47.1	30.7	46.1	32.0	34.6	14.3			Reference	Reference
Vaccinated, %	51.1	52.9	69.3	53.9	68.0	65.4	85.7			−0.18	0.01
Clinical flu, *n*	50	65	99	89	61	28	7	0.02	4.79*		
Not vaccinated, %	46.0	46.2	32.3	43.8	32.8	39.3	14.3			Reference	Reference
Vaccinated, %	54.0	53.8	67.7	56.2	67.2	60.7	85.7			−0.14	0.03
Antiviral status[6], *n*											
Confirmed flu, *n*	46	53	88	78	50	26	7	<0.01	< 0.01		
No prescribed antivirals, %	13.0	13.2	12.5	15.4	10.0	19.2	0.0			Reference	Reference
Prescribed antivirals, %	87.0	86.8	87.5	84.6	90.0	80.8	100			−0.004	0.96
Clinical flu, *n*	54	66	98	93	60	28	7	<0.01	0.08		
Not prescribed antivirals, %	13.0	16.7	15.3	17.2	15.0	17.9	0.0			Reference	Reference
Prescribed antivirals, %	87.0	83.3	84.7	82.8	85.0	82.1	100			−0.02	0.78

[1] Values are mean ± SD, number, or percentage. *$P < 0.05$, ***$P < 0.001$.
[2] Data were collected from November, 2008 to February, 2009.
[3] 1 cup = 200 mL.
[4] Of the 204 pupils analyzed for confirmed influenza, 16 responses for d/wk and 42 for cup/d were missing. On the other hand, of the 241 pupils analyzed for clinical influenza, 20 responses for d/wk and 50 for cup/d were missing.
[5] Absent due to illness only.
[6] The antiviral status of neuraminidase inhibitors such as oseltamivir (Tamiflu) or zanamivir (Relenza) for the treatment of influenza infection.

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TABLE 5 Regression analysis of the days of absence from school following influenza infection and green tea consumption (d/wk) for elementary schoolchildren in Kikugawa City

			Green tea consumption (d/wk)						
			<3[1]	3 to <6			≥6		
	R^2	*F*	*n*	*n*	*β*	*P*	*n*	*β*	*P*
Confirmed influenza									
Simple regression	0.04	4.34*	47	52	−0.25	0.005	87	−0.20	0.03
Multiple regression[2]	0.11	2.85**	42	49	−0.27	0.004	81	−0.17	0.07
Clinical influenza									
Simple regression	0.05	5.38**	55	65	−0.26	0.002	98	−0.21	0.01
Multiple regression[2]	0.23	8.24***	46	61	−0.20	0.02	91	−0.10	0.25

[1] Reference group.
[2] Adjusted for all potential confounding variables that are listed in Table 4: age, sex, school absence days before this survey, influenza vaccination status, and antiviral status. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

tea compared with <1 cup/d of green tea (**Table 3**). In the multivariate logistic regression model 1, the adjusted OR inversely associated with the consumption of 1 to <3 cups/d and 3–5 cups/d compared with <1 cup/d were 0.62 [(95% CI = 0.41–0.95); *P* = 0.03] and 0.54 [(95% CI = 0.30–0.94); *P* = 0.03], respectively, in the case of confirmed influenza, and 0.65 [(95% CI = 0.43–0.97); *P* = 0.03] and 0.54 [(95% CI = 0.31–0.92); *P* = 0.02], respectively, in the case of clinical influenza. The results of model 2 were in agreement with those of model 1. However, the consumption of >5 cups/d of green tea was not associated with the incidence of influenza infection in any of the regression models.

Green tea consumption and days of absence from school following influenza infection. The mean length of absence from school because of influenza infection was 3–4 d. Antiviral drugs for influenza treatment were prescribed to >80% of patients at all green tea consumption levels (**Table 4**). Regression analyses for the days of absence from school following influenza infection and each confounding factor revealed that influenza vaccination appeared to be somewhat effective in terms of decreasing the length of absence from school following influenza infection; however, the R^2 value was very low (β coefficient: −0.18, *P* = 0.01, R^2 = 0.03 for confirmed influenza; β coefficient: −0.14, *P* = 0.03, R^2 = 0.02 for clinical influenza).

Multiple regression analysis for the days of absence from school following influenza infection and the frequency of green tea consumption (d/wk) showed that the consumption of green tea for 3 to <6 d/wk inversely affected the days of absence from school following influenza infection compared with the consumption of green tea for <3 d/wk in clinical and confirmed influenza; however, the R^2 value was low (β coefficient: −0.27, *P* = 0.004, R^2 = 0.11 in confirmed influenza; β coefficient: −0.20, *P* = 0.02, R^2 = 0.23 in clinical influenza) (**Table 5**). Multiple regression analysis for the days of absence from school following influenza infection and the quantity of green tea consumption (cups/d) revealed no association for any level of green tea consumption (**Table 6**).

Discussion

The findings of our observational study reveal that the consumption of 1–5 cups of green tea on an almost daily basis (i.e. ≥6 d/wk) is inversely associated with the incidence of influenza infection in elementary schoolchildren.

These findings are supported by previous research, which documented the prevention of influenza infection via the antiviral effects of green tea catechins and the enhancement of systemic immunity of theanine (20–26). Experimental studies have demonstrated that tea catechins bind to the hemagglutinin molecule of the influenza virus and also inhibit viral adsorption to Madin-Darby canine kidney cells, thus providing an insight into the mechanisms by which tea catechin extracts inhibit the influenza virus (20–22,27). Rowe et al. (25) reported that the consumption of tea catechins and theanine enhances systemic immunity ($\gamma\delta$ T-cell function) and prevents the occurrence of

TABLE 6 Regression analysis of the days of absence from school following influenza infection and green tea consumption (cups/d) for elementary schoolchildren in Kikugawa City

			Green tea consumption (cups/d)[1]									
			<1[2]	1 to <3			3–5			>5		
	R^2	*F*	*n*	*n*	*β*	*P*	*n*	*β*	*P*	*n*	*β*	*P*
Confirmed influenza												
Simple regression	0.02	1.28	78	50	−0.16	0.05	25	−0.04	0.64	7	−0.03	0.73
Multiple regression[3]	0.08	1.55	71	49	−0.14	0.12	23	−0.02	0.86	7	0.01	0.92
Clinical influenza												
Simple regression	0.02	1.18	93	61	−0.14	0.06	27	−0.04	0.60	7	−0.04	0.59
Multiple regression[3]	0.24	6.35***	81	59	−0.09	0.24	25	0.01	0.84	7	0.02	0.83

[1] 1 cup = 200 mL.
[2] Reference group.
[3] Adjusted for all potential confounding variables, which are listed in Table 4; age, sex, school absence days before this survey, influenza vaccination status, and antiviral status. ***$P < 0.001$.

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cold and flu symptoms in healthy adults. Furthermore, the application of green tea catechins as alternative antiinfluenza viral agents has been suggested (28). Recently, Kuzuhara et al. (29) reported that green tea catechins inhibit the endonuclease activity of influenza A virus RNA polymerase and that their galloyl group is important for this function; docking simulations revealed that catechins with a galloyl group stably bound to the active pocket of the endonuclease domain. These results could facilitate the refining and optimization of catechin-based drug designs with increased stability. The antiinfluenza effects of strychnine and caffeine, which are components of green tea, have also been documented (30–32).

Interestingly, we found that the consumption of >5 cups/d (1000 mL) of green tea was not associated with the incidence of influenza infection in elementary schoolchildren. However, the number of children who consumed >5 cups/d of green tea was much lower than that of the other groups and it is possible that this reduced the statistical validity. Green tea is recognized as a healthy beverage worldwide and its safety for human consumption is supported by the fact that Asians have been drinking it for ~1000 y. Nevertheless, the harmful effects of overconsumption of green tea have also been reported. According to a recent review (33), liver damage caused by a high level of green tea consumption (or concentrated green tea extracts) has been found to occur. The reviewers suggested that the ingestion of concentrated green tea extracts along with food minimizes the possible risk of liver damage; however, they also noted that this proposal does not pertain to traditional green tea infusions or other beverage preparations. Caffeine is a component of green tea, and caffeine toxicity in children is manifested by severe emesis, tachycardia, central nervous system agitation, and diuresis. Furthermore, chronic exposure to caffeine has been implicated in a range of dysfunctions involving the gastrointestinal, liver, renal, and musculature systems (34). Considering these observations together, the adverse effects of excessive consumption may have affected the immunity of the children included in our survey. A further large-scale study should be performed to assess the safety of green tea consumption by children.

The geographical region where we conducted our study is one of the highest tea-producing regions of Japan. Adults and children living in this area are accustomed to drinking green tea after each meal, not only at home but also at school. The levels of daily green tea consumption for the children included in our survey were approximately the same as those for average Japanese adults (35,36), possibly because of this specific geographical circumstance. We collected green tea samples from 8 randomly extracted families from different schools and analyzed the quantities of primary bioactive components (i.e. catechins and caffeine) using HPLC (37). We found that the mean concentrations of total catechins and caffeine were 137 ± 66.8 mg/cup (range, 56.6–272 mg) and 36.5 ± 21.4 mg/cup (range, 14.0–79.2 mg), respectively (M. Park, H. Yamada, K. Matsushita, T. Goto, Y. Okada, and T. Kitagawa, unpublished data). Although the proportions of bioactive components varied widely in each sample, it is clear that some children may have consumed extremely high amounts of catechins and caffeine. Warzak et al. (38) suggested that the caffeine content of green tea would cause sleep disturbance in children at consumption quantities of 1–5 cups/d. In our survey, the children included did not manifest sleep disturbance (Table 1) despite consuming high caffeine concentrations. It is possible that these children had induced caffeine resistance due to growing up in a high tea-producing region.

The days of absence from school following influenza infection showed a tendency to decrease in relation to the frequency (d/wk) of green tea consumption; however, the R^2 value was very low. It is possible that the days of absence from school were not always in accordance with the recovery period. We excluded from our analysis any child who was infected with influenza during a holiday or winter vacation. However, it is possible that the number of days of absence from school were fewer than the actual influenza-affected period, because of holidays or winter vacation days being included in the affected period. A further interventional study should be performed, with accurate assessment of the number of affected days and degree of symptoms, to examine the reduction in influenza symptoms by green tea consumption.

Our study had certain limitations. First, the data for the preventive measures (i.e. the frequency and, particularly, the quantity of green tea consumption) contained several omissions in reporting. It may have been difficult for pupils or their parents to recall their activities over a 2-mo period and to make an accurate assessment of consumption; therefore, the questionnaire may have been difficult to complete. Second, we were not able to exclude some bias, i.e., whether respondents were systematically different from nonrespondents (i.e. interest for participation in the study or intelligence of parents and children), and how much the children's answers were influenced by the parents' participation. Third, the benefit of green tea consumption for influenza infection was accessed by multivariate regression models adjusted for plausible confounding factors (one of which, the risk of influenza infection by household transmission, was very strong). However, there may be other confounding factors that we did not anticipate, because the setting of this survey was restricted to a tea plantation area of Japan, the environment of which differs from that of other regions. Finally, contrary to the results of green tea consumption, general preventive measures (such as influenza vaccination, hand hygiene, and the use of facemasks) were not associated with the incidence of influenza infection. Paradoxically, ventilation was associated with the incidence of influenza infection. Based on this result, it would be helpful to include a more accurate definition of "ventilation" in the survey questions.

In conclusion, our findings suggest that the consumption of 1–5 cups/d of green tea may prevent influenza infection in elementary schoolchildren. However, our results may be restricted to the participating children living in a specific geographical region of Japan. Further clinical studies, including randomized controlled trials, are required to confirm the preventive effects of green tea consumption on influenza infection, including the number of affected days and degree of symptoms, as well as to assess the safety of green tea consumption by children.

Acknowledgments

M.P. and H.Y. designed the research, participated in the study coordination, and wrote the paper; M.P. and S.K. were responsible for the integrity of the data and the accuracy of the data analysis; and K.M., T.G., Y.O., K.K., and T.K. participated in the study coordination and the collection and analysis of the data. All authors read and approved the final manuscript.

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