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

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)

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.

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.

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.

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, schoo 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. ***$P < 0.001$.

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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Effects of Short-Term Consumption of a Large Amount of Tea Catechins on Chromosomal Damage, Oxidative Stress Markers, Serum Lipid, Folic Acid, and Total Homocysteine Levels : A Randomized, Double-Blind, Controlled Study

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Objective : To evaluate the effects of short-term consumption of a large amount of tea catechins on chromosomal damage, oxidative stress markers, serum lipid, folic acid, and total homocysteine levels in middle-aged healthy volunteers.
Methods : Forty volunteers (40-63 years) participated in a randomized, double-blind study. After a 1-week washout, the catechin group consumed approximately 1069 mg/day of total catechins for 1 week. The micronucleated binucleate cells (MNi) frequency in the cytokinesis-block micronucleus cytome assay, urinary 8-hydroxydeoxyguanosine (8-OHdG), isoprostane, lymphocyte and plasma vitamin C, serum lipid, folic acid, and total homocysteine levels were measured at the beginning and end of the intervention.
Results : No significant differences were observed between the catechin and placebo groups in terms of MNi frequency, urinary 8-OHdG, isoprostane, or lymphocyte and plasma vitamin C levels. The serum LDL-cholesterol level in the catechin group significantly decreased compared with pre-intervention period, and there was a decreased tendency in the catechin group compared with the placebo group, but the difference was not significant ($P=0.105$). The serum folic acid level decreased ($P=0.073$) and the total homocysteine level significantly increased in the catechin group ($P=0.029$). No serious adverse events were observed during the study.
Conclusions : A large amount of tea catechins, which corresponds to approximately 10 cups of green tea per day for 1 week, seemed to be well tolerated, and did not influence chromosomal damage and the oxidative stress markers. Further long-term and large-scale studies are required to clarify the long-term effect of the consumption of a large amount of tea catechins on these markers as well as on improving dyslipidemia.
Trial Registration : ClinicalTrials.gov ID, NCT00448513

Key words : catechins, chromosomal damage, oxidative stress, LDL-cholesterol, dyslipidemia

Introduction

Tea has been consumed since ancient times and is one of the most popular beverages around the world, second to water. About 80% of the tea production worldwide is black tea, which is the main tea beverage consumed in Europe and North America. Green tea, which is mainly consumed in Asian countries such as China, Korea, and Japan. In the processing of green tea fresh leaves, the leaves are steamed or pan-dried at a high temperature right after plucking, resulting in minimal oxidation of the naturally occurring

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catechins in the tea leaves. In general, the amount of catechins in green tea is relatively high, up to 30% of their dry weight. The major catechins are (+)-catechin (C), (−)-epicatechin (EC), (+)-gallocatechin (GC), (−)-epigallocatechin (EGC), (−)-catechin gallate (Cg), (−)-epicatechin gallate (ECg), (−)-gallocatechin gallate (GCg), and (−)-epigallocatechin gallate (EGCg)[1]. In green tea, particularly, a high amount of EGCg constitutes approximately 50 to 60% of the total tea catechins.

A considerable amount of scientific research on tea has been conducted over the past several decades[2,3], and it has revealed that tea catechins exert various physiological effects that are beneficial to human health, such as antioxidative effect[4-7], anticarcinogenic effect[8], reduction of dyslipidemia[9-13], antiobesitic effect[14], reduction of high blood pressure[15], antibacterial effect[16], antiviral effect[17], and antiallergic effect[18]. With regard to the antioxidative effect, Sugisawa et al.[6] reported in an *in vitro* study that physiological concentrations of EGCg less than 1 μmol/L are not genotoxic, but can prevent reactive oxygen species-induced chromosomal damage. Hakim et al.[7] reported in a clinical study that after 4 months of drinking 4 cups of decaffeinated green tea per day, urinary 8-hydroxydeoxyguanosine (8-OHdG), which is one of the oxidative stress markers, significantly decreased. With regard to reduction of dyslipidemia, Koo and Noh[12] reported that green tea or catechins inhibited the intestinal absorption of dietary lipids. Davies et al.[13] reported that 5 cups of tea per day for 3 weeks reduced the total and LDL cholesterol levels. According to well-controlled designs, however, there is little clinical evidence of chromosomal damage, antioxidative effect or reduction of dyslipidemia after a large amount consumption of purified tea catechin extracts.

Among the green tea components, catechins may be important with respect to obtaining an antioxidant effect or reducing dyslipidemia. Therefore, more rapid and greater effects would be obtained if large amounts of catechins or purified catechins are administered. On the background, a randomized, double-blind, controlled study was performed to evaluate the influence of short-term consumption of a large amount of tea catechins on chromosomal damage, oxidative stress markers, and serum lipid levels in middle-aged healthy volunteers.

Materials and Methods

Subjects

Volunteers of either sex, who were between 40 and 65 years of age, were recruited from the local area of Shizuoka city where the research was performed. The intervention started in November 2007 and ended in August 2008. Volunteers were excluded if they were taking any medications or drugs, if they had allergies to tea or catechins, if they were pregnant women, or if they were taking any dietary supplements containing antioxidants (e.g., vitamin E) or folic acid in the previous 3 months. They were also prohibited from taking these supplements during the entire course of the study.

The study was approved by the ethics committee of the University of Shizuoka and Shizuoka General Hospital, in accordance with the Declaration of Helsinki. Written informed consent was obtained from the subjects before entering the study.

Design and Intervention

The study was a randomized, double-blind, placebo-controlled, parallel-group design with 1 week of intervention preceded by a 1-week washout period. After the 1-week washout period, the subjects were randomly divided into 2 groups, namely, the catechin group and the placebo group, by using computer-generated random numbers. Randomization was carried out with a stratified block method, and sex and smoking status were selected as stratified factors. During the intervention period, the subjects took 3 capsules containing either catechins or placebo 3 times a day for 1 week. The subjects were instructed to avoid taking the test capsules with meals, and they took the capsules at certain intervals of 30 minutes or more. During the 2 weeks including washout and intervention periods, the subjects were not allowed to drink any tea beverages containing catechins or take any supplements that might affect either their oxidative stress markers or serum lipids. The subjects were also instructed not to make any changes to their usual food intake, alcohol consumption, or physical activity.

Usual tea drinking, alcohol consumption, smoking habit, and physical activity were assessed by a questionnaire at the baseline of random allocation. The subjects were asked to record in their diaries any test capsules taken, any adverse events, or any

concomitant medications or supplements taken.

Test Substances

THEA-FLAN 90S (ITO EN, LTD., Tokyo, Japan), containing about 70% gallated catechin, was used as the test capsule for intervention. The catechin content was measured with high-performance liquid chromatography (HPLC)[19]. The total catechin content per 3 capsules was 356.48 mg, including 270 mg EGCg, 6.78 mg GCg, 71.39 mg ECg, and 4.09 mg EGC. In the present study, the subjects allocated to the catechin group took a total amount of 1069.44 mg catechins per day. The placebo capsules were made from inert ingredients and were identical to the active capsules in color, appearance, weight, and odor.

Outcome Measures

All measurements were performed before and after the intervention period. Blood and urine samples were taken in the early morning after the subjects had fasted overnight. Chromosomal damage in the peripheral blood lymphocytes was assayed using the cytokinesis-block micronucleus (CBMN) method[20,21] for cultured lymphocytes. Briefly, heparinized blood from each subject was divided into two parts in culture bottles. Whole blood (0.5 mL each) was added to 4.5 mL of RPMI-1640 culture medium supplemented with 10% fetal calf serum and 1% antibiotics. The culture was immediately initiated by adding 0.1 mL of phytohemagglutinin. Cytochalasin B (final concentration 5.4 μg/mL) was added at 44 hours to induce binucleated cells. The cells were harvested at 72 hours and treated with hypotonic media for more than 2 min. Slides were prepared using a cytocentrifuge (Shandon Southern Products : Cheshire, UK). The slides were air-dried for 60 min, fixed with absolute methanol, and then stained with 4% (v/v) Giemsa's solution in water for 30 min. Chromosomal damage rates were expressed as the number of micronucleated binucleate cells (MNi) per 1000 binucleated cells.

The measurements of urinary 8-OHdG and urinary isoprostane levels were analyzed by enzyme immunoassay methods. For the measurements of vitamin C, lymphocytes and plasma were prepared by centrifugation and the Ficoll gradients method, then immediately treated with metaphosphoric acid (final 5% wt/wt) to stabilize vitamin C[22]. These processes were performed within 2 h under cooled conditions on ice to obtain reliable data. The vitamin C samples were stored at −80℃ until analysis, and the vitamin C (ascorbic acid, reduced form) levels were measured by HPLC with the electrochemical detector method[23]. All samples were handled and stored similarly in both groups.

EGCg and epigallocatechin-3-*O*-(3-*O*-methyl)-gallate (EGCg3"Me) in blood samples was prepared with ethyl acetate and analyzed by HPLC equipped with a coulochem II electrochemical detector (ESA, Inc., Bedford, MA)[24].

Other blood and urine samples were analyzed by SRL, Inc. independently. The parameters analyzed included total cholesterol, LDL-cholesterol (LDL-cho), HDL-cholesterol (HDL-cho), triacylglycerol (TG), folic acid, total homocysteine, AST, ALT, γ-GTP, lactate dehydrogenase, total bilirubin, creatinine, total protein, albumin, sodium, potassium, chloride, red blood cell count, white blood cell count, hemoglobin, hematocrit, and platelet count.

Safety Monitoring

Adverse events were collected from all subjects who took at least 1 test capsule and were included in the safety analysis. Clinical laboratory blood and urine tests were performed and blood pressure and pulse rates were measured during the pre- and post-intervention period for safety monitoring.

Statistical Analyses

The observed values for each measurement and change (value observed in the post-intervention minus that in the pre-intervention : Δ values) were represented by mean (SD). The baseline characteristics of the 2 groups were compared by Fisher's exact test for categorical variables and an unpaired t test. The significance of any inter-group difference in the changes was tested by an unpaired t test. The correlation between the changes in the MNi frequency and the levels of catechins was tested by Pearson's correlation coefficient test. Intra-group comparisons of the data obtained post-intervention period versus pre-intervention were tested by the paired t test. Intergroup comparisons were tested by analysis of covariance to determine if the differences between the groups were significantly different. In each analysis, a P value<0.05 was considered to be statistically significant. A safety evaluation, such as adverse events and abnormal findings from clinical

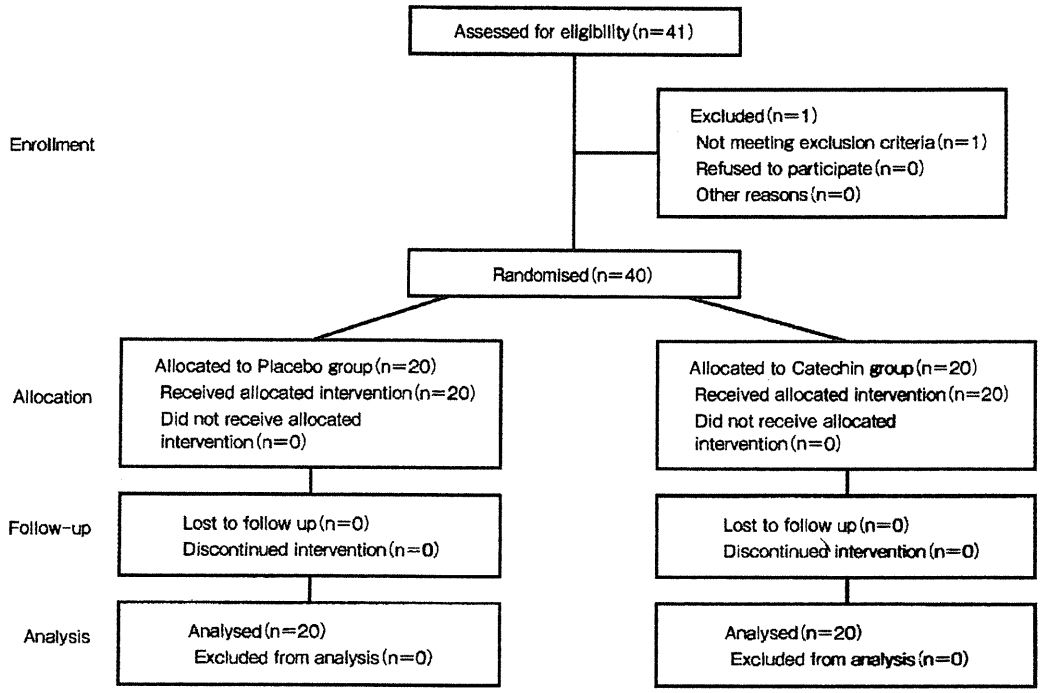

Fig. Flow chart of the study

laboratory tests, was performed using Fisher's exact test and an unpaired *t* test. All statistical analyses were performed using R for Windows release 2.6.2 (R Development Core Team, 2008).

Results

The study flow chart is shown in Figure. Forty-one volunteers were recruited. On the basis of the exclusion criteria, a male volunteer was excluded because of hepatic dysfunction, leaving 40 subjects to be allocated randomly to the catechin group or the placebo group. All 40 subjects completed the study, and there were no withdrawals. Adherence with the test capsules was 98.3% in the catechin group and 98.6% in the placebo group.

The baseline characteristics are presented in Table 1. All 40 subjects (17 men, 23 women), were Japanese, aged between 40 and 63 years (49±6.7 years), and had a mean body mass index of 21.1 kg/m^2. The average of the subjects' usual tea drinking was 591 mL/day, which corresponded to approximately 2 PET plastic bottles of commercial green tea in Japan. No significant differences were observed among the 2 groups with respect to the values of the parameters shown in Table 1. The plasma levels of catechins (EGCg, EGCg3"Me) increased significantly in the catechin group compared with that in the placebo group (ΔEGCg, $P<0.001$: ΔEGCg3"Me, $P<0.001$, Table 2).

The chromosomal damage and oxidative stress marker analysis data for each group are shown in Table 3. An assessment of the MNi frequency in the peripheral blood lymphocytes did not show a significant difference between the groups ($P=0.924$). There was no significant correlation between the change in MNi frequency and plasma catechins (EGCg, $r=0.028$, $P=0.864$: EGCg3"Me, $r=0.075$, $P=0.645$). Also there were no significant differences in urinary 8-OHdG, isoprostane, lymphocyte and plasma vitamin C levels between the 2 groups.

The serum lipid, folic acid, and total homocysteine analyses data for each group are shown in Table 4. LDL-cho was significantly decreased by 7.6% post-intervention compared with pre-intervention in the catechin group ($P=0.007$), and there was a decreased tendency in the catechin group compared with the placebo group (Table 4, $P=0.105$). In contrast, the TG level increased by 24.5% ($P=0.104$) in the catechin group, and the TG level of the catechin group was significantly different from that of the placebo group ($P=0.019$).

Serum folic acid level was significantly decreased

Table 1 Baseline characteristics of subjects in the placebo and catechin groups

	All subjects n=40	placebo n=20	catechin n=20	*P* value
Age (year)	49±6.7	49±6.9	49±6.7	0.963
Sex (Men/Women)	17/23	9/11	8/12	1.000
BMI (kg/m^2)	21.1±2.7	21.3±2.7	20.8±2.7	0.557
Tea drinking (mL/day)	591±359	671±433	519±269	0.453
MNi frequency (‰)	15.8±7.7	16.7±8.0	14.9±7.5	0.335
8-OHdG (ng/mg CRE)	4.6±2.9	5.0±3.0	4.3±2.9	0.402
Isoprostane (pg/mg CRE)	248.0±513.6	167.1±51.2	328.9±724.7	0.892
Lymphocyte vitamin C (nmol/mg protein)	22.6±5.8	22.8±6.0	22.4±5.7	0.655
Plasma vitamin C (μmol/L)	54.6±13.4	55.7±15.2	53.5±11.6	0.989
EGCg (μg/mL)	0.017±0.034	0.010±0.026	0.025±0.040	0.118
T-cho (mg/dL)	207.2±28.6	205.9±29.9	208.5±28.0	0.778
HDL-cho (mg/dL)	66.7±14.7	65.8±13.2	67.7±16.4	0.681
LDL-cho (mg/dL)	121.9±27.7	121.4±29.3	122.5±26.7	0.902
TG (mg/dL)	93.2±55.5	97.4±69.1	89.1±38.9	0.968
Folic acid (ng/mL)	5.8±2.1	6.1±2.4	5.5±1.7	0.285
Total homocysteine (nmol/mL)	8.8±3.1	9.4±3.7	8.2±2.3	0.402

Data are expressed as mean (SD) or number. None of the differences between the groups were statistically significant.

Table 2 Plasma levels of catechins

Variable	Study group	Pre-intervention (PRE)	Post-intervention (POST)	Delta (POST-PRE, Δ)	*P* value (Difference between groups)
EGCg (μg/mL)	placebo	0.03±0.04	0.01±0.03	−0.01±0.04	<0.001*
	catechin	0.01±0.03	0.12±0.06	0.12±0.07	
EGCg3"Me (μg/mL)	placebo	0.00±0.00	0.00±0.00	0.00±0.00	<0.001*
	catechin	0.00±0.00	0.08±0.03	0.08±0.03	

placebo group (n=20), catechin group (n=20). Δ : delta values were calculated by subtracting the value of PRE from the value of POST.
* : $P<0.001$, compared with placebo.

Table 3 Effects of tea catechins on oxidative stress markers

Variable	Study group	Pre-intervention (PRE)	Post-intervention (POST)	*P* value (Difference within groups)	Delta (POST-PRE, Δ)	*P* value (Difference between groups)
MNi frequency (‰)	placebo	16.7±8.0	19.0±9.2	0.239	2.3±7.8	0.924
	catechin	14.9±7.5	17.5±9.7	0.144	2.5±6.9	
8-OHdG (ng/mg CRE)	placebo	5.0±3.0	3.9±1.7	0.055	−1.1±2.5	0.313
	catechin	4.3±2.9	4.2±3.3	0.409	−0.1±4.3	
Isoprostane (pg/mg CRE)	placebo	167.1±51.2	194.6±113.8	0.332	9.2±42.5	0.463
	catechin	328.9±724.7	298.3±529.2	0.648	−30.7±220.6	
Lymphocyte vitamin C (nmol/mg protein)	placebo	22.8±6.0	19.9±4.1	0.022†	−2.9±5.0	0.641
	catechin	22.4±5.7	20.3±6.7	0.057	−2.1±4.3	
Plasma vitamin C (μmol/L)	placebo	55.7±15.2	50.7±14.5	0.083	−5.0±12.2	0.121
	catechin	53.5±11.6	53.8±15.6	0.882	0.3±7.6	

placebo group (n=20), catechin group (n=20). Δ : delta values were calculated by subtracting the value of PRE from the value of POST.
† : $P<0.05$, compared with PRE (PRE vs POST).

Table 4 Effects of tea catechins on serum lipid, folic acid, and total homocysteine levels

Variable	Study group	Pre-intervention (PRE)	Post-intervention (POST)	*P* value (Difference within group)	Delta (POST-PRE, Δ)	*P* value (Difference between groups)
Total-cho (mg/dL)	placebo	205.9±29.9	202.2±27.9	0.269	−3.7±14.5	0.356
	catechin	208.5±28.0	200.4±26.0	0.351	−8.2±14.7	
HDL-cho (mg/dL)	placebo	65.8±13.2	65.6±13.7	0.912	−0.2±6.0	0.442
	catechin	67.7±16.4	66.1±17.8	0.286	−1.7±5.5	
LDL-cho (mg/dL)	placebo	121.4±29.3	119.4±28.2	0.503	−2.0±13.1	0.105
	catechin	122.5±26.7	113.2±23.1	0.007††	−9.3±13.7	
TG (mg/dL)	placebo	97.4±69.1	82.1±51.1	0.086	−15.3±41.7	0.019*
	catechin	89.1±38.9	110.8±54.0	0.104	21.8±52.9	
Folic acid (ng/mL)	placebo	6.1±2.4	6.3±2.6	0.563	0.2±1.4	0.073
	catechin	5.5±1.7	4.9±2.2	0.043†	−0.5±1.1	
Total homocysteine (nmol/mL)	placebo	9.4±3.7	9.7±4.0	0.896	0.3±1.6	0.029*
	catechin	8.2±2.3	9.6±2.7	<0.001†††	1.4±1.2	

placebo group (n=20), catechin group (n=20). Δ : delta values were calculated by subtracting the value of PRE from the value of POST.
† : $P<0.05$, †† : $P<0.01$, ††† : $P<0.001$, compared with PRE (PRE vs POST). * : $P<0.05$, compared with placebo.

by 9.1% post-intervention compared with pre-intervention in the catechin group ($P=0.043$), and there was a decreased tendency between the 2 groups ($P=0.073$). The total homocysteine level was significantly increased by 17.1% post-intervention compared with pre-intervention in the catechin group ($P<0.001$), and there was a significant difference between the groups ($P=0.029$).

No serious adverse events including laboratory change were observed during the study. In both groups, digestive symptoms appeared as an adverse event, but were relativity mild (placebo group, soft feces, n=1 ; catechin group, constipation, n=2). There were no significant differences in adverse events or laboratory results between the catechin and placebo groups.

Discussion

It is generally known that DNA and chromosomal damage are responsible for the initiation and evolution of cancer and for the acceleration of aging[21,25]. The MNi frequency of the CBMN assay, which is one of the main products of chromosomal damage, is measured as a reliable high-sensitive genotoxic marker, and has recently been attempted to be used as an oxidative stress marker[21]. We evaluated the MNi frequency in middle-aged healthy subjects and observed no significant differences between the catechin and placebo groups. Therefore, the results showed that chromosomal damage and genotoxicity were not accelerated within a week of consuming a large amount of tea catechins.

Urinary 8-OHdG is known to be the main product of the oxidative damage of DNA, and urinary isoprostane is known to be one of the main products of the oxidative damage of cell membrane[26]. Vitamin C is known to be an antioxidant and can be measured as an index of antioxidative potential[27]. To measure vitamin C level, lymphocyte vitamin C was considered to reflect the state of vitamin C stockpiling in tissues, and was not easily influenced by diet or daily circadian fluctuation[28]. We evaluated the antioxidative effect by using the measurements of urinary 8-OHdG, isoprostane, and lymphocyte and plasma vitamin C levels, and showed no significant differences between the groups. The results might suggest that these oxidative stress markers were not affected during the short intervention period of a week, with the amount of tea catechins used.

With regard to lipid regulation, LDL-cho in the catechin group significantly decreased compared with pre-intervention period, and there was a decreased tendency in the catechin group compared with the placebo group, but the difference was not significant ($P=0.105$). There were no significant differences between the groups in terms of either total or HDL-cho levels. Conversely, the TG level significantly increased in the catechin group. The serum TG level

was influenced by various factors such as the recent intake of food, beverage, and alcohol ; therefore, the results need to be carefully interpreted. We calculated the total energy and lipid levels in the diet of each participant and found that these amounts widely varied in each individual and in each period as follows : The median total energy level was 1765 kcal/day for the pre-intervention period (range, 453-2985 kcal/day) and 1744 kcal/day (range, 931-3002 kcal/day) for the intervention period. The median total lipid level was 55 g/day (range, 11-136 g/day) for the pre-intervention period and 54 g/day (range, 11-122 g/day) for the intervention period. However, it was reported that lipoprotein lipase (LPL), which degraded TG to non-esterified fatty acid and glycerol, was inhibited by tea catechins[29], which may indicate a possible mechanism of TG elevation. However, it was also reported that tea catechins inhibited the absorption of TG in the small intestines, and decreased the serum TG levels[9]. Further clinical studies should be performed with well-controlled diets for a few days before blood sampling to elucidate the effect of tea catechins on the serum TG levels.

Serum folic acid showed a tendency to decrease with the consumption of tea catechins. Tea catechins are reported to inhibit conjugase, an enzyme involved in the absorption of folic acid, in the small intestine epithelium[30]. Therefore, the results support the report that the absorption of folic acid was inhibited by tea catechins. Moreover, the total homocysteine level significantly increased by consuming tea catechins. The serum homocysteine level is influenced by folic acid, vitamin B6, and vitamin B12 ; of these, folic acid is considered to be the main factor influencing the serum homocysteine level. There was also a negative correlation between folic acid and homocysteine levels[31]. It was thought that the total homocysteine level increased because of the decrease in folic acid level in the catechin group. The results imply that folic acid should be added to the diet when large amounts of catechins are being consumed. In daily tea drinking, however, where folic acid is contained in green tea, the decrease in folic acid by catechins may not have any influence on human health.

Our study has certain limitations. First, the duration of intervention was relatively shorter than that used in published studies on the effectiveness of consuming large amounts of catechins[7,13]. However, the amount of catechins used in our study corresponds to consuming approximately 10 cups of green tea per day and is almost 2-fold higher than the amount reported by Hakim et al[7] and Davies et al[13] (4-5 cups per day). The safety of using such large amounts of catechins in humans has not yet been well established[32] ; therefore, we investigated the effects of only short-term consumption in order to ensure the safety of the participants. Furthermore, we determined the amounts of catechins on the basis of the effects of EGCg on plasma superoxide scavenging activity, at 2 hours after oral ingestion of catechins[33]. However the effectiveness of catechins on our outcome measures might be underestimated because of the short study periods. Second, a small sample size was used. Although the sample size was determined on the basis of feasibility, the statistical power seemed to be weakened with respect to confirming the effectiveness of catechins. For clarifying the effects of these limitations, additional long-term and large-scale randomized trials should be performed.

In conclusion, a large amount of tea catechins, which corresponds to approximately 10 cups of green tea per day for 1 week, seemed to be well tolerated, and did not influence chromosomal damage and the oxidative stress markers. Further long-term and large-scale studies are required to clarify the long-term effect of the consumption of a large amount of tea catechins on these markers as well as on improving dyslipidemia.

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Conflict of Interest

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短 報

健康食品摂取に伴う健康被害報告の因果関係評価法の構築：改定評価票による評価者間信頼性評価

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Study on the Evaluation Approach of Causal Relationships between Health Food Products and Adverse Events : Application of the Revised Evaluation Scale for Inter-Rater Reliability

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緒 論

近年，消費者の健康志向の高まりから健康食品の需要が増加し，それに伴い健康食品の摂取に伴う健康被害事例が報告されるようになってきている[1,2]．健康食品の摂取に伴う健康被害が生じた場合，その情報はドラッグストアや製造販売元への利用者からの相談や，医療機関で治療を受けた場合の診療録等を基に，保健所を介して厚生労働省に集約されていく．しかし，その情報は種々雑多であり，正確に把握し因果関係の評価判定を行うことは極めて難しい．また，そのような情報を基にして因果関係を科学的に吟味するための臨床上有用な方法論も十分に確立していない[1~3]．

すでに医薬品の投与に伴う有害事象の因果関係評価においては，Naranjo らの評価票や Jones の評価アルゴリズム等，種々の判定法が報告されている[4~6]．我々はこれまでに，健康食品と医薬品が共に機能的に生体に作用するという類似性に着目し，Naranjo らの評価票や Jones の評価アルゴリズム等を改変することで，健康食品の摂取に伴う健康被害の因果関係評価法の構築を試みてきた[3,7]．その過程で，Naranjo らの評価票の改変が健康食品に適用しやすい可能性を示したものの，評価者間信頼性評価においていまだ十分とは言えず，たとえば製品の品質が必ずしも保証されていない問題など，医薬品とは異なる健康食品の特性を考慮した改定が必要という課題が残された．本研究では以上の背景を踏まえ，すでに作成した評価票の質問項目や点数の重み付け等を再検討し改定することで，より評価者間信頼性の高い評価法の構築を試みた．

方 法

すでに作成した Naranjo ら改変評価票[7]を再検討し，健康食品の摂取により生じた有害事象の因果関係判定に必要と考えられる必須基本情報，健康食品の特性を考慮した質問項目や点数の重み付け，さらに合計点による評価判定の基準について吟味し，加筆や修正を加えた（Fig. 1A, 1B）．改定は，以下の 4 点について行った．1）摂取した健康食品の必須基本情報として，商品名，製造者，主成分・含有量，1 日摂取目安量等を問う項目を加えた．2）Naranjo ら改変評価票における「プラセボが与えられたとき，その有害事象は起こりましたか？」と「血中（或いは他の体液）の濃度が毒性域に入っていましたか？」という 2 つの質問項目を削除した．3）点数の重み付けに関して，「当該健康食品を再摂取した際，有害事象はまた現れましたか？」と「その有害事象は客観的証拠によって確かめられましたか？」という 2 つの質問項目について，1 点ずつ点数を加算した．4）Naranjo ら改変評価票で possible にまとめられていた評価を，新たに highly possible を加えることで 2 つに分け，合計点による評価判定を 4

Key words : health food, adverse event, causal relationship, inter-rater reliability
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1A：健康食品の必須背景情報

記入日　　年　　月　　日

疑わしい健康食品が複数ある場合は、用紙を替えてそれぞれについて評価を行ってください。

疑わしい健康食品等について

商品名	
製造者	
主成分・含有量	
1日摂取目安量	
摂取量	
摂取期間	から　　まで

生じた有害事象について

有害事象名	
発生期間	から　　まで
重篤な有害事象ですか？	はい　・　いいえ

重篤な有害事象とは以下のものです（各申 GCP 2-15*より抜粋）。

* 医薬品の臨床試験の実施の基準（GCP）の内容（中央薬事審議会答申）より引用。
（URL: http://www.mhlw.go.jp/shingi/2007/04/dl/s0420-8r.pdf）

- 死亡に至るもの
- 生命を脅かすもの
- 治療のため入院若しくは入院・加療期間の延長が必要なもの
- 永続的若しくは重大な障害・機能不全に陥るもの
- 先天異常を来たすもの
- その他の重大な医学的事象

摂取者の情報

性別	男性　・　女性
年齢	歳
併用健康食品	
併用医薬品	

1B：質問項目

評価票

有害事象を評価するために以下の質問に答え、適切な点数をつけてください

No.	質問項目	はい	いいえ*1	わからない
1.	生じた有害事象は、当該健康食品の添付文書やラベルに記載されているものですか？	+1	0	0
2.	当該健康食品を摂取した後に、有害事象が現れましたか？	+2	-1	0
3.	当該健康食品を中止した際、有害事象は改善されましたか？	+1	0	0
4.	当該健康食品を再摂取した際、有害事象はまた現れましたか？	+3	-1	0
5.	その有害事象を引き起こすかもしれない（当該健康食品以外の）他の要因*2はありますか？	-1	+2	0
6.	その有害事象は摂取量を増量したとき程度は重くなり、減量したとき軽くなりましたか？	+1	0	0
7.	以前に、同じかあるいは類似の健康食品または医薬品で同様の有害事象が現れましたか？	+1	0	0
8.	その有害事象は客観的証拠*3によって確かめられましたか？	+2	0	0

合計点

*1 「いいえ」という答えは、どのような代替案を考慮したとしても、十分な情報が存在しない場合を前提とします（不確かなとき、あるいは情報不足で評価できない場合は、「わからない」としてください）。

*2 他の要因としては、基礎疾患や合併症の病態、併用薬や他の健康食品の摂取、年齢などを考慮します。

*3 客観的証拠とは、当該健康食品に含まれる成分に対して DLST、パッチテストなどの特異的な検査によって確認されたものです。

合計点による評価判定

9≤	非常に確からしい（highly probable）
5–8	確からしい（probable）
3–4	可能性が強くある（highly possible）
1–2	可能性が弱くある（possible）
≤0	ほぼ関連なし（doubtful）

本評価票で「わからない」という回答が多い場合は、情報量不足により評価できない（詳細不明である）ことを意味します。

Fig. 1　健康食品の因果関係評価票（改定版）

段階から5段階評価とした.

次いで改定した評価票およびすでに作成したNaranjoら改変評価票を用い，健康食品販売業者のお客様センターに寄せられた保健機能食品(特定保健用食品，栄養機能食品）および保健機能食品以外のいわゆる健康食品の摂取に伴う健康被害相談事例200例に対して，6カ月の評価間隔をあけ，5人の評価者〔薬学部学生3名，薬学研究科大学院生1名および（独)国立健康・栄養研究所認定の栄養情報担当者（NR）有資格者1名〕により，それぞれ独立に因果関係を評価した．その後，得られた評価票を回収し，評価点の合計に基づきカテゴリー分類した．評価者間信頼性の評価として，多評価者間 κ 係数と級内相関係数（ICC：intraclass correlation coefficient）を算出した．なお，本研究で利用した健康被害相談事例の個別内容については，機微情報を含むことから提示しないこととした．

結　果

改定した評価票を用い因果関係を評価した結果，200例の評価のうち，5名の評価者ともhighly probableに分類した事例はなく，probable，highly possible，possibleおよびdoubtfulのいずれかに分類された(Fig. 2)．多評価者間 κ 係数とICC［95%信頼区間］はそれぞれ，0.77と0.77［0.73，0.81］となった．一方，すでに作成したNaranjoら改変評価票の多評価者間 κ 係数とICC［95%信頼区間］はそれぞれ，0.12と0.33［0.27，0.40］となった．

考　察

本研究では，これまでに我々が作成してきた評価票と改定した評価票の信頼性を，同じ評価者と評価事例を適用して比較検討した．その結果，改定した評価票では，これまでに作成した評価票と比べ，κ 係数，ICC共に高い値を示し，評価者間信頼性の向上が認められた．調査票の改定にあたっては医薬品と異なる健康食品の特性が反映されるように，まず質問項目に関しては，プラセボ投与や血中濃度測定といった，健康食品では情報が得られにくい質問を削除した．次いで点数の重み付けに関しては，質問項目が減ったことによる点数の低下を防ぐことも考慮し，再投与による症状の再現や客観的証拠の存在といった因果関係がより強くあると判断できる項目の配点を高くした．また，これまでの研究結果において[3,7]，健康被害事例の因果関係判定が低いカテゴリーに集中する傾向が示されていたことから，possibleの分類を二分した．今回の結果に

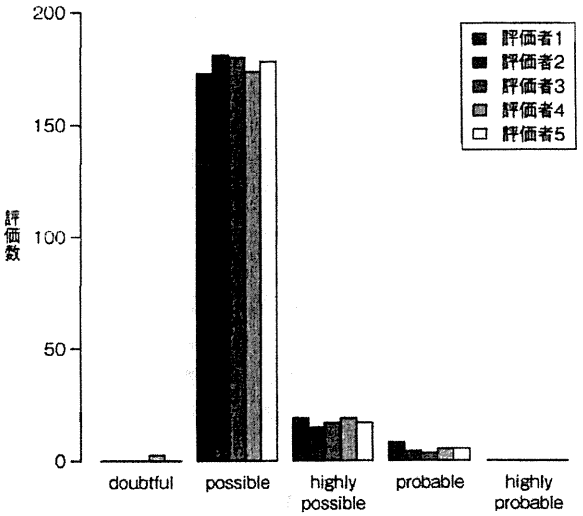

Fig. 2　評価結果の分布（評価者5名，有害事象事例200例）

おいてもpossibleに分類される事例が多かったが，これは因果関係評価に必要な情報が不足している事例が多かったためだと考えられる．しかし，改定した評価票では，より情報が多いと考えられる一部の事例が，新たに設けたカテゴリーであるhighly possibleに篩い分けできた．これらの工夫により，より健康食品の特性に合う改定となり，かつ主観的判断の影響も入りにくくなった結果，評価者間信頼性が向上したと考えられた．

因果関係判定の信頼性評価においては，得られる情報が正確かつ十分であることが，評価の質に大きく影響する．その点，健康食品から得られる情報は医薬品と比べ不十分で，判断が難しいことが多いと指摘されている[1~3,7]．しかしながら，そのような判断が難しい事例こそ，健康被害の原因究明のために因果関係の判定が最も求められている状況もあり得る．判断が難しい事例では，製品の品質や含有量に関する情報が不明瞭であったり，製品表示と内容物が一致していなかったり，あるいは複数の健康食品素材が含まれているといったラベル表示の段階での情報不足の場合があり，このような状況下では因果関係の評価は困難と言わざるを得ない．そのため今回改定した評価票では，健康食品の必須基本情報として最低限必要と思われる情報の有無をまず確認し，その時点で情報不足であれば，評価前に調査をし直すことを促せるようにした．

今回改定した評価票の質問項目は，情報不足のため「わからない」と回答した場合，その質問項目での得点は0点となる．そのため，実際に因果関係が少ない事

例と情報量不足のため評価不能とすべき評価事例が，カテゴリー分類を見ただけでは判別できない事態が起こり得る．そのような場合には，回答が「わからない」となる項目の合計が一定数以上の場合，評価不能とするといった選り分けが必要であるが，本評価票ではその対応も十分可能である．すなわち，この改定評価票は，多様な健康被害報告のモニタリングやスクリーニングに有用と考えられる．

今後，この評価票を用い，臨床現場で正しくかつ効率的に因果関係を評価するためには，臨床現場で遭遇する健康食品の摂取によるさまざまな健康被害報告に対して評価票を適用し，妥当性の検討を行う必要があると考える．

結　論

本研究では，これまでに我々が作成した健康食品の摂取に伴う健康被害の因果関係評価票を健康食品の特性を考慮し改定することで，より信頼性の高い評価票を作成することができた．今後，評価票の妥当性評価を含め，臨床現場での有用性を検証していく必要がある．

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