Actual Daily Intakes of Tea Catechins and Their Estimation According to Four Season 3 Day Weighed Dietary Records and a Short Food Frequency Questionnaire among Japanese Men and Women

Kaori Endoh¹, Yuji Matsui², Masao Takeshita², Mitsuhiro Katashima², Koichi Yasunaga², Kiyonori Kuriki¹*

Abstract

Background: Tea catechins are considered to be important preventive factors of cancer on several organs; however, the relationships of the actual daily intakes (ADIs) on the preventive effects have not been adequately addressed. We measured the ADIs of tea catechins as annual averages derived from every their ingested cups recorded by each subject, and the estimation models were established considering tea origin. Methods: Fifty-nine Japanese men and women completed four season 3 day weighed dietary records (WDRs) and a food frequency questionnaire (FFQ), and samples of green, oolong and black teas, ingested during a total 12 days were collected for the analysis. The ADIs of the total and composed catechins of all tea samples were measured by a high-performance liquid chromatography. The estimation models for the ADIs (R²: coefficient of determination) based on the WDRs and FFQ were established with multiple regression analysis using appropriate confounding factors. Results: The ADIs of total catechins and epigallocatechin gallate (EGCg) were 110 and 21.4 mg/day in men and 157 and 34.7 mg/day in women, respectively. The total catechins ADIs were positively associated with green tea consumption based on WDRs and FFQ (adjusted R²=0.421 and 0.341 for men and 0.346 and 0.238 for women, p<0.05 for all, respectively). Likewise, the EGCg ADIs were associated with green tea intake derived from WDRs and FFQ, respectively. Conclusions: We revealed the ADIs of total catechins and EGCg as annual averages could establish their estimation models. These provide reference information to clarify their relationships with cancer risks.

Keywords: Tea catechins- actual daily intake- food frequency questionnaire- green tea- estimation model

Introduction

As cancer is one of the leading causes of death worldwide, reducing the cancer burden is a major global public health goal. Tea has been suggested to have preventive effects against cancer and is a popular beverage, especially in Asia. Green, black, and oolong teas are all derived from Camellia sinensis and represent major sources of catechins in Japan, China, and the UK, in that order (Otaki et al., 2009; Knaze et al., 2012).

Catechins have been shown to inhibit stomach cancer in vitro and vivo (Zhu et al., 2007); however, the associations between tea and cancer risks on several organs including gastric and bladder cancer, are inconsistent (Sasazuki et al., 2004; Wu et al., 2013). Epigallocatechin gallate (EGCg), a major component of catechins, has anti-cancer effects such as antioxidant properties and induces cell cycle arrest (Yu et al., 2014). The levels of catechins in the leaves of dried teas vary according to period of storage (Lee et al., 2008). Furthermore, the levels of catechins in brewed tea vary depending on the brewing methods such as differences in water temperature, even if the same volume of the dried leaves is used. Thus, the concentrations of tea and catechins are thought to differ substantially both between- and within-subjects. However, the actual daily intakes (ADIs) of tea and catechins have not yet been investigated because of the difficulty in collecting each tea sample ingested for each study subject for study period. Such information is necessary for conducting epidemiological studies of the ADIs of catechins to help clarify their potential biological and cancer preventive effects and the most effective and responsible dose.

Toward this end, the aim of the present study was to reveal the ADIs of total catechins and epigallocatechin gallate (EGCg), which were determined by high-
performance liquid chromatography with ultraviolet detection (HPLC-UV) from tea samples collected by Japanese subjects after each ingestion during four season 3 day weighed dietary records (WDRs). Based on these data, estimation models of the ADIs among Japanese men and women were established to serve as reference data for further epidemiological studies on their cancer preventive effects.

Materials and Methods

Study participants

This study was part of the Japan Multi-Institutional Collaborative Cohort Study (J-MICC Study) Sakura Diet Study (J-MICC SDS) conducted from January 2013 to March 2014. The participants were residents of Shizuoka City, Japan, as described previously (Hisada et al., 2015). In brief, 58 men and 29 women participated in the J-MICC SDS, representing a subpopulation of the Shizuoka-Sakuragakao Study, which was the member of J-MICC Study (Hamajima, 2007; Naito et al., 2008). We verbally explained the purpose of the study to the participants and obtained their written informed consent from all individuals prior to inclusion. Lifestyle data, including dietary habits (Tokudome et al., 2005), were collected using a self-administered questionnaire. We asked the participants to collect the tea samples ingested and complete 12d WDRs (3-day WDRs in each of the four seasons of the year) and two food frequency questionnaires (FFQs: FFQ1 and FFQ2) before and after the 12d WDRs (Figure 1). Finally, 38 men and 21 women completed all requirements and were included in the present analysis. This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving participants were approved by the ethics committee of the University of Shizuoka (No. 24-24).

Tea sampling and records

The participants were asked to collect a portion (approximately 10 mL) of each cup of tea ingested during the 12d WDRs. They recorded the volume, time, place, and variety of tea: green tea [Sencha (steamed tea leaves, the standard green tea), Bancha (made from hardened tea leaves or coarse Sencha), Gyokuro (fine quality green tea that is grown in the shade), Genmaicha (Sencha mixed with roasted rice), Houjicha (roasted tea leaves), or other], black tea, oolong tea, bottled green tea, green tea extract, or catechins supplement. The WDRs were systematically checked by trained dieticians. The participants also completed FFQs both before and after the 12d WDRs. These FFQs contained additional questions regarding the habitual intake of green, black, and oolong teas with the following answer choices (allocated daily frequency is indicated in the parentheses): green tea [never or seldom (0), 1–3 times/month (0.1), 1–2 times/week (0.2), 3–4 times/week (0.5), 5–6 times/week (0.8), once/day (1.0), twice/day (2.0), or 3+ times/day (3.0)] and black and oolong teas [never or seldom (0), 1–3 times/month (0.1), 1–2 times/week (0.2), 3–4 times/week (0.5), 5–6 times/week (0.8), 1–2 times/day (1.5), 3–4 times/day (3.5), or 5+ times/day (5)]. The portion size of each cup for daily intake was estimated according to the data of (Saito et al., 2015) as follows: green tea, 140 mL/cup for men and 130 mL/cup for women; black tea, 150 mL/cup; oolong teas 120 mL/cup.

Catechins and caffeine analysis of tea samples

Caffeine and eight individual catechins (EGCg, epicatechin gallate, epigallocatechin, epicatechin, gallatechin gallate, catechin gallate, gallochin, and catechin) from each tea sample were analyzed using HPLC-UV (Guillarme et al., 2010). The sum of eight catechins is presented as the total catechins content. In the case of commercial bottled green tea, we obtained the same brand listed and used the purchased sample for analysis. The ADIs of total tea catechins, EGCg, and caffeine were calculated by multiplying the analyzed concentration and the reported daily intake. The inter-assay coefficients of variation (CV) for concentrations of catechins, EGCg, and caffeine were 1.5%, 4.4%, and 1.3%, respectively.

Statistical analysis

Among the 38 men and 21 women (67.8% of all participants) included in the analysis, we obtained 708 WDRs. The sample size was estimated using “pwr.f2.test” of the R package “pwr”. Based on a numerator degrees of freedom of 6, effect size of 0.15, type I error probability of 0.05, and power of 0.9, the calculated sample size was 97.3. The age [mean ± standard deviation (SD)] was 45.9 ± 8.7 and 39.8 ± 8.0 years in men and women, respectively, and the body mass index (BMI) was 23.2 ± 2.9 and 20.5 ± 3.0 kg/m², respectively. Green tea extract was not reported in any of the WDRs, whereas catechins supplements were reported in 8.6% (n = 61) of the WDRs; these were excluded from the analyses.

Total tea intake was defined as the sum of green, black, oolong, and bottled green teas. Means, SDs, CVs, minimum, maximum, median, skewness, and kurtosis of green, black, oolong, bottled green, and total tea, and the ADIs of total catechins, EGCg, and caffeine were calculated. Green tea and bottled green tea were analyzed separately because the catechins levels of brewed and bottled green tea are different (Mizukami et al., 2007). With reference to previous studies (Kim et al., 2013), we calculated variance based on 1) subjects (between-subjects), 2) season, 3) recording sequence, 4) day of the week (weekday or weekend) and 5) residual (within-subject). The ratios of within-subject to between-subject variance components were estimated by the variance ratio (VR) ([within-subject variance (σ²b)]/between-subject variance (σ²w)).

The associations between the ADIs of total catechins, EGCg, and caffeine from daily tea consumption based on 12d WDRs were calculated for all subjects and for men and women, separately. Multiple linear regression analyses were conducted with the ADIs of total catechins, EGCg, or caffeine as dependent variables and daily tea consumption (green tea, black tea, or oolong tea), BMI, age, and sex (men = 0; women = 1) as independent variables. For BMI, body weight was measured once in each season. We also analyzed daily tea consumption based on the FFQ1 and FFQ2. The first and fourth BMI...
values were used as independent variables in the analysis for FFQ1 and FFQ2, respectively. All statistical analyses were conducted using R 3.3.0 (Comprehensive R Archive Network, https://cran.r-project.org/) using the “e1071”, “lmerTest”, and “pwr” packages. All p values were based on two-tailed tests. P values < 0.05 were statistically considered significant.

Results

Women more frequently consumed Sencha, Genmaicha, Houjicha, and black tea, whereas men more frequently consumed Bancha, other green teas, oolong tea, and bottled green tea (supplemental Table 1).

Table 1 shows the basic statistics regarding the consumption of the different teas as well as the ADIs of total catechins, EGCG, and caffeine in all tea samples during the 12d WDRs. This table also shows the results of the two FFQs. In some cases, the minimum value is given as 0, which indicates the days on which the participants did not drink any tea. Overall, the ADIs of total catechins, EGCG and caffeine were highest in women than in men.

Table 2 shows the contribution of the variation for the intake of each types of tea to the ADIs of total catechins, EGCG, and caffeine among Japanese men and women. In both sexes, the largest source of variation was within-subject variation for total catechins and between-subject variation for total tea intake. In both sexes, the VRs of total catechins were > 1.0 and the VRs of total tea were < 1.0. Other sources of variation, such as season, recording sequence and day of week, contributed less than 10% to the total, except for Genmaicha and Houjicha in men and oolong tea in women.

Table 3 and supplemental tables 2 shows results of the multiple regression analysis, including coefficients of determination (R²), for the ADIs of total catechins, EGCG, and caffeine intake from tea as dependent variables and daily tea intake based on the 12d WDRs and FFQ1 as independent variables, with sex, age, and BMI as confounding factors. In both men and women, the ADIs of total catechins, EGCG, and caffeine were positively associated with green tea (p<0.01 for all) derived from the 12d WDRs and FFQ1. In men only, the ADIs of total catechins were also positively associated with oolong tea (p<0.05) based on both 12d WDRs and FFQ1. There was no association of the total catechins ADI with black tea. The same result for the ADI of EGCG was obtained when pooling the data of men and women together (supplemental tables 2). When bottled green tea was included, the adjusted R² values for the ADIs of total catechins, EGCG, and caffeine were 0.484, 0.295, and 0.696 in all participants; 0.529, 0.357, and 0.735 in men; and 0.345, 0.184, and 0.651 in women, respectively. Overall, the ADIs of total catechins, EGCG, and caffeine were most strongly significantly associated with green and bottled green tea intake derived from 12d WDRs.

Discussion

We firstly demonstrated that the ADIs of tea and total catechin show substantial between- and within-individual variation. In the previous studies, the ADIs of total catechins and EGCG were calculated by multiplying the reported consumption frequency or consumed quantity according to the assigned standard catechins content of teas (Arts et al., 2001). These values might not be precise because of the wide variation in the concentrations and total catechins contents in teas. The results of the ADIs of total catechins and EGCG obtained in the present study can therefore be considered to be more accurate since we collected a portion of every ingested cup during the 12-day WDRs from the subjects and directly determined the concentrations of total catechins and EGCG by HPLC-UV for all tea samples collected during the year.

The ADIs of total catechins and EGCG were positively associated with green tea based on both 12d WDRs and the FFQ. Therefore, we demonstrated that the ADIs of total catechins and EGCG estimated from the regression equation derived from 12d WDRs and FFQs was almost equivalent to the individual total catechins and EGCG consumption considering each tea source. This variation might explain the inconsistency of previous epidemiological studies on the relationships between tea and cancers such as stomach and bladder cancer (Sasazuki et al., 2004; Wu et al., 2013), given that the beneficial effect of tea on cancers is derived from the catechins.

In Asia, the population is ageing more quickly than in other regions. Given the increase in cancer prevalence and the number of cancer-related deaths with age, the beneficial effects of catechins on cancer prevention might be expected to be more evident in Asian populations where tea is a very popular non-alcoholic beverage. This represents an important research area since cancer is one of the leading causes of death worldwide.

Consumer trends in Japan for green tea have changed in recent years, with the annual consumption of green tea leaves per family decreasing by 28.1% from 2001 to 2015 (Ministry of Agriculture, 2016). In contrast, the annual consumption of bottled green tea per person increased from approximately 11 L to 19.4 L during this period. In the present study, the adjusted R² for ADIs was approximately the same or higher when including bottled green tea as an independent variable, implying that bottled green tea contributes to the habitual daily intake of the ADIs of total catechins, EGCG, and caffeine. Therefore, bottled green tea should be considered along with green tea in studies regarding the association with disease.

This study has several limitations. First, the results are not generalizable because the study participants were not selected randomly. Second, we did not consider catechins intake from other sources except tea such as fruits and
Table 1. Distribution of Tea and Measured Total Catechins, Epigallocatechin Gallate (EGCg), and Caffeine in All Tea Samples based on 12d Weighed Food Records (WDRs) and Food Frequency Questionnaires (FFQs) among Japanese Men and Women

|                      | Mean  | SD   | CV (%) | Minimum | Median | Maximum | Skewness | Kurtosis |
|----------------------|-------|------|--------|---------|--------|---------|----------|----------|
| 12d WDRs             |       |      |        |         |        |         |          |          |
| Green tea (mL/day)   | 283   | 393  | 139    | 0       | 150    | 2,380   | 1.9      | 4.3      |
| Sencha (mL/day)      | 192   | 327  | 171    | 0       | 0      | 2,380   | 2.4      | 7.7      |
| Bancha (mL/day)      | 23.7  | 120  | 506    | 0       | 0      | 891     | 5.4      | 28.3     |
| Gyokuro (mL/day)     | 0.4   | 9.4  | 2,350  | 0       | 0      | 200     | 21.2     | 449      |
| Genmaicha (mL/day)   | 2.3   | 30.6 | 1,330  | 0       | 0      | 600     | 17.1     | 321      |
| Houjicha (mL/day)    | 0.8   | 16.4 | 2,050  | 0       | 0      | 350     | 21.2     | 449      |
| Other green tea (mL/day) | 73.7  | 191  | 259    | 0       | 0      | 1,794   | 4.2      | 23.1     |
| Black tea (mL/day)   | 13.4  | 67.0 | 500    | 0       | 0      | 600     | 5.7      | 35.0     |
| Oolong tea (mL/day)  | 32.6  | 128  | 393    | 0       | 0      | 810     | 4.3      | 18.4     |
| Bottled green tea (mL/day) | 49.2 | 148  | 301    | 0       | 0      | 1,000   | 3.3      | 11.4     |
| Total tea (mL/day)   | 430   | 459  | 107    | 0       | 300    | 2,380   | 1.4      | 2.0      |
| ADI of Total catechins (mg/day) | 110 | 173  | 157    | 0       | 26.3   | 1,600   | 2.9      | 13.9     |
| ADI of EGCg (mg/day) | 21.4  | 41.6 | 194    | 0       | 1.6    | 350     | 3.4      | 15.0     |
| ADI of Caffeine (mg/day) | 47.9 | 65.0 | 136    | 0       | 22.3   | 455     | 2        | 5.2      |

| FFQ1                  |       |      |        |         |        |         |          |          |
| Green tea (mL/day)    | 263   | 145  | 55.3   | 0       | 280    | 420     | -0.3     | -1.4     |
| Black tea (mL/day)    | 16.6  | 28.9 | 174    | 0       | 7.5    | 120     | 2.5      | 6.1      |
| Oolong tea (mL/day)   | 34.7  | 93.8 | 270    | 0       | 12     | 420     | 3.6      | 12.0     |

| FFQ2                  |       |      |        |         |        |         |          |          |
| Green tea (mL/day)    | 250   | 155  | 62.1   | 0       | 280    | 420     | -0.2     | -1.5     |
| Black tea (mL/day)    | 24.1  | 53.3 | 221    | 0       | 0      | 225     | 2.9      | 8.0      |
| Oolong tea (mL/day)   | 24.6  | 55.1 | 224    | 0       | 0      | 180     | 2.3      | 3.7      |

| Women (n = 21)        |       |      |        |         |        |         |          |          |
| 12d WDRs              |       |      |        |         |        |         |          |          |
| Green tea (mL/day)    | 373   | 342  | 91.6   | 0       | 300    | 1,650   | 0.9      | 0.4      |
| Sencha (mL/day)       | 318   | 335  | 105    | 0       | 200    | 1,500   | 1.0      | 0.3      |
| Bancha (mL/day)       | 2.7   | 25.4 | 941    | 0       | 0      | 265     | 9.5      | 90.8     |
| Gyokuro (mL/day)      | 1.4   | 22.0 | 1,571  | 0       | 0      | 350     | 15.7     | 245      |
| Genmaicha (mL/day)    | 8.0   | 54.2 | 678    | 0       | 0      | 600     | 8.2      | 74.4     |
| Houjicha (mL/day)     | 10.3  | 61.9 | 601    | 0       | 0      | 550     | 7.1      | 52.7     |
| Other green tea (mL/day) | 39.9 | 124  | 311    | 0       | 0      | 800     | 3.7      | 14.6     |
| Black tea (mL/day)    | 74.7  | 159  | 213    | 0       | 0      | 1,000   | 2.4      | 6.5      |
| Oolong tea (mL/day)   | 32.8  | 130  | 397    | 0       | 0      | 750     | 4.0      | 15.1     |
| Bottled green tea (mL/day) | 30.6 | 114  | 374    | 0       | 0      | 530     | 3.6      | 11.5     |
| Total tea (mL/day)    | 548   | 381  | 69.5   | 0       | 515    | 1,650   | 0.3      | -0.6     |
| ADI of Total catechins (mg/day) | 157 | 173  | 111    | 0       | 106    | 867     | 1.4      | 2.0      |
| ADI of EGCg (mg/day)  | 34.7  | 48.0 | 138    | 0       | 17.9   | 279     | 2.2      | 5.7      |
| ADI of Caffeine (mg/day) | 71.0 | 68.2 | 96.1   | 0       | 52.3   | 306     | 0.9      | 0.1      |

| FFQ1                  |       |      |        |         |        |         |          |          |
| Green tea (mL/day)    | 300   | 110  | 36.5   | 65.0    | 390    | 390     | -0.7     | -0.9     |
| Black tea (mL/day)    | 72.9  | 124  | 169    | 0       | 15.0   | 525     | 2.4      | 5.8      |
| Oolong tea (mL/day)   | 38.3  | 90.3 | 236    | 0       | 12.0   | 420     | 3.6      | 12.2     |

| FFQ2                  |       |      |        |         |        |         |          |          |
| Green tea (mL/day)    | 287   | 128  | 44.8   | 26.0    | 390    | 390     | -0.7     | -1.0     |
| Black tea (mL/day)    | 75.7  | 122  | 162    | 0       | 30.0   | 525     | 2.5      | 5.9      |
| Oolong tea (mL/day)   | 53.1  | 124  | 233    | 0       | 12.0   | 420     | 2.4      | 4.4      |

ADI, actual daily intake; CV, coefficient of variation; EGCg, epigallocatechin gallate; SD, standard deviation; Total catechins are summed based on the following compounds: EGCg, epicatechin gallate, epigallocatechin, epicatechin, galloatechingallate, catechingallate, galloatechin, and catechin. b Daily consumption of the ADIs of total catechin, EGCg, and caffeine were calculated using the measured concentrations and multiplying by the total intake. Frequency of the dietary intake of green, black, and oolong tea was assessed as described in the Methods. c Daily tea intake (cups/day) was determined from FFQs. The FFQs contained additional questions regarding the habitual intake of green, black, and oolong teas as described in the Methods.
Asian Pacific Journal of Cancer Prevention, Vol 18

**Actual and Estimated Catechins Intakes**

Third, there were relatively few participants, especially women. Indeed, the VRs of the ADIs of total catechins and EGCg were much higher among women than among men. Fourth, we recruited participants living in Shizuoka, which is a famous green tea-cultivating area in Japan; thus, the results might not be representative of other areas. Indeed, the mean daily intake of green tea was 282.5 mL in men and 373.3 mL in women, which are higher than those reported for the general Japanese population, with a mean intake of green, black, and oolong teas of 247.9 mL in men and 266.3 mL in women (Ministry of Health, 2016). Fifth, we did not consider other potential confounding factors such as past or present history of disease or socioeconomic status. Finally, 32.2% of the participants did not complete sufficient 12-day WDRs and these tea samples were excluded from the analyses.

In conclusion, we revealed the ADIs of total catechins and EGCg, and the results indicated that the individual ingestion of tea catechins can be properly estimated using statistical methods. These data should prove useful for investigating the associations with cancer risks on several organs in further large-scale observational studies.

**Conflict of interest disclosure**

Yuji Matsui, Masao Takeshita, Mitsuhiro Katashima, and Koichi Yasunaga are employees of Kao Corporation.

**Table 2. Contribution of Variation (%) for Each Tea to the ADIs of Total Catechins, Epigallocatechin Gallate, and Caffeine among Japanese Men and Women**

|                     | Between-subject (subject, σ²) | Season (subject, σ²) | Recording sequence (residual, σ²) | Day of the week (residual, σ²) | Within-subject (residual, σ²) | Variance ratio (σ²/σ²) |
|---------------------|-------------------------------|----------------------|----------------------------------|-------------------------------|-----------------------------|---------------------|
| **Men (n = 38)**    |                               |                      |                                  |                               |                             |                     |
| Green tea (mL/day)  | 69.5                          | 0.6                  | 0                                | 2.8                           | 27.1                        | 0.39                |
| Sencha (mL/day)     | 73.7                          | 0.5                  | 0                                | 0.0                           | 25.8                        | 0.35                |
| Bancha (mL/day)     | 66.1                          | 2.5                  | 0                                | 0.0                           | 31.3                        | 0.47                |
| Gyokuro (mL/day)    | 0                             | 0.2                  | 0                                | 0.0                           | 99.8                        | NA                  |
| Genmaicha (mL/day)  | 0                             | 0                    | 0.0                             | 53.6                          | 46.4                        | NA                  |
| Houjicha (mL/day)   | 0                             | 0                    | 0.0                             | 61.4                          | 38.6                        | NA                  |
| Other green tea (mL/day) | 37.1                      | 0                    | 0                               | 0.0                           | 62.9                        | 1.69                |
| Bottled green tea (mL/day) | 33.4                      | 0                    | 0                               | 0.0                           | 66.6                        | 1.99                |
| Black tea (mL/day)  | 16.4                          | 0.4                  | 0                                | 0.0                           | 83.2                        | 5.09                |
| Oolong tea (mL/day) | 42.5                          | 0                    | 0                               | 0.0                           | 57.5                        | 1.35                |
| Total tea (mL/day)  | 67.6                          | 0.3                  | 2.1                             | 0.0                           | 29.9                        | 0.44                |
| ADI of total catechins (mg/day) | 35.3                   | 0                    | 0.4                             | 0.0                           | 64.3                        | 1.82                |
| ADI of EGCg (mg/day) | 42.9                          | 0                    | 0.4                             | 0.0                           | 56.7                        | 1.32                |
| ADI of caffeine (mg/day) | 61.0                        | 0                    | 0.0                             | 0.0                           | 39.0                        | 0.64                |
| **Women (n = 21)**  |                               |                      |                                  |                               |                             |                     |
| Green tea (mL/day)  | 57.0                          | 0.2                  | 0                                | 0.0                           | 42.8                        | 0.75                |
| Sencha (mL/day)     | 54.1                          | 0.5                  | 0                                | 0.0                           | 45.4                        | 0.84                |
| Bancha (mL/day)     | 81.5                          | 7.5                  | 0                                | 0.0                           | 11.1                        | 0.14                |
| Gyokuro (mL/day)    | 0                             | 0.2                  | 0.0                             | 0.0                           | 99.8                        | NA                  |
| Genmaicha (mL/day)  | 53.8                          | 0                    | 0.7                             | 0.0                           | 45.6                        | 0.85                |
| Houjicha (mL/day)   | 35.9                          | 0                    | 0.0                             | 0.0                           | 64.1                        | 1.78                |
| Other green tea (mL/day) | 25.2                        | 0.9                  | 0.0                             | 0.0                           | 73.9                        | 2.93                |
| Bottled green tea (mL/day) | 33.6                      | 0                    | 0                               | 0.0                           | 66.4                        | 1.98                |
| Black tea (mL/day)  | 37.6                          | 0.1                  | 0.0                             | 0.0                           | 62.3                        | 1.66                |
| Oolong tea (mL/day) | 65.7                          | 0.2                  | 0.0                             | 10.0                          | 24.1                        | 0.37                |
| Total tea (mL/day)  | 57.9                          | 0.4                  | 4.5                             | 0.0                           | 37.2                        | 0.64                |
| ADI of total catechins (mg/day) | 27.9                   | 3.2                  | 0.4                             | 0.0                           | 68.5                        | 2.46                |
| ADI of EGCg (mg/day) | 2.7                            | 5.8                  | 0.4                             | 0.0                           | 91.2                        | 33.9                |
| ADI of caffeine (mg/day) | 49.7                            | 0                    | 0.3                             | 0.0                           | 50.0                        | 1.01                |

ADI, actual daily intake; EGCg, epigallocatechin gallate; NA, not available; a, Total catechins were summed from the following compounds: epigallocatechin gallate, epicatechingallate, epigallocatechin, epicatechin, gallocatechin gallate, catechingallate, gallocatechin, and catechin; b, The values of ADI of total catechin, EGCg, and caffeine were calculated by using the measured concentrations and multiplying by the total intake.
Table 3. Multiple Linear Regression Analyses of Daily Intakes of Measured Catechins, Epigallocatechin Gallate, and Caffeine among Japanese Men and Women

| Independent variables | Men | Women |
|-----------------------|-----|-------|
| Age (years)           | 2.43** 0.296 0.772*** -0.589 -0.418 0.26 | 1.40 0.008 -0.589 0.282 0.451*** 0.071 |
| BMI (kg/m²)           | 1.73 0.412 0.347 5.47 1.21 1.71 | 0.140*** 0.008 0.114 0.639*** -0.416 -0.056 |
| Daily green tea intake (mL/d) | 0.254*** 0.052*** 0.102*** 0.303*** 0.067*** 0.140*** | 0.325*** -11.9 0.102*** 0.016 0.346*** 0.238 |
| Daily black tea intake (mL/d) | 0.149 0.043 0.068* 0.025 0.001 0.136*** | -49.6 0.024 0.347 3.38 1.22 0.052*** |
| Daily oolong tea intake (mL/d) | 0.233*** 0.070*** 0.215*** 0.066 0.005 0.138*** | 0.237* 0.26 0.296 |
| Intercept              | -122 -19.3 -32.1 -49.6 1.41 -41.8 | 122; 12; -105 -10 -11.9 -198 -34.2 -60.8 |
| Adjusted R²            | 0.421*** 0.325*** 0.666*** 0.346*** 0.200*** 0.639*** | 0.341** 0.128 0.451*** 0.238 0.163 0.296 |

Based on 12d WDRs

Based on FFQ1

\*p < 0.05; **p < 0.01; ***p < 0.001; ADI, actual daily intake; EGCg, epigallocatechin gallate; FFQ, food frequency questionnaire; WDR, weighed dietary record. Example of the regression equation for ADI of total catechin (mg/day) of men: ADI of total catechins = 2.43 × Age + 1.73 × BMI + 0.254 × Daily green tea intake based on 12d WDRs + 0.149 × Daily black tea intake base on 12d WDRs + 0.233 × Daily oolong tea intake based on 12d WDRs - 122; c. Based on the 12d WDRs, the values of total catechins, EGCg, and caffeine were calculated based on the concentration of catechins, EGCg, and caffeine by volume and summed as daily intakes. Based on FFQ1, catechins, EGCg, and caffeine of 12d WDRs were averaged and used as dependent variables; d. Total catechin was summed based on the following: EGCg, epicatechin gallate, epigallocatechin, epicatechin, gallatechin gallate, catechin gallate, gallatechin, and catechin; e. Daily tea intake (cups/day) was counted from 12d WDRs; f. Daily green tea was summed as total daily green tea (Sencha, Bancha, Gyokuro, Genmaicha, Houjicha, and other green tea); g. Daily tea intake (cups/day) was counted from FFQ1. The FFQ contained additional questions regarding the habitual intake of green, black, and oolong teas as described in the Methods.

Acknowledgements

This work was supported by Grants-in-Aid for Scientific Research on Priority Areas of Cancer (No. 17015018), Innovative Areas (No. 221S0001), JSPS KAKENHI Grant Number JP (No. 16H06277) and Research C (No. 26350154) from the Japanese Ministry of Education, Culture, Sports, Science and Technology.

References

Arts IC, Hollman PC, Feskens EJ, et al (2001). Catechin intake might explain the inverse relation between tea consumption and ischemic heart disease: the Zutphen Elderly Study. Am J Clin Nutr, 74, 227-32.

Arts IC, van de Putte B, Hollman PC (2000). Catechin contents of foods commonly consumed in The Netherlands. 1. Fruits, vegetables, staple foods, and processed foods. J Agric Food Chem, 48, 1746-51.

Guillarme D, Casetta C, Bicchi C, et al (2010). High throughput qualitative analysis of polyphenols in tea samples by ultra-high pressure liquid chromatography coupled to UV and mass spectrometry detectors. J Chromatogr A, 1217, 6882-90.

Hamajima N (2007). The Japan multi-institutional collaborative cohort study (J-MICC Study) to detect gene-environment interactions for cancer. Asian Pac J Cancer Prev, 8, 317-23.

Hisada T, Endoh K, Kuriki K (2015). Inter- and intra-individual variations in seasonal and daily stadibilities of the human gut microbiota in Japanese. Arch Microbiol, 197, 919-34.

Kim DW, Kyung Park M, Kim J, et al (2013). Sources of variation in nutrient intake and the number of days to assess usual intake among men and women in the Seoul metropolitan area, Korea. Br J Nutr, 110, 2098-107.

Knaze V, Zamora-Ros R, Lujan-Barroso L, et al (2012). Intake estimation of total and individual flavan-3-ols, proanthocyanidins and theaflavins, their food sources and determinants in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Br J Nutr, 108, 1095-108.

Lee VS, Dou J, Chen RJ, et al (2008). Massive accumulation of gallic acid and unique occurrence of myricetin, quercetin, and kaempferol in preparing old oolong tea. J Agric Food Chem, 56, 7950-6.

Mizutani Y, Sawada Y, Yamaguchi Y (2007). Simultaneous analysis of catechins, gallic acid, strictinin, and purine alkaloids in green tea by using catechol as an internal standard. J Agric Food Chem, 55, 4957-64.

Ministry of Agriculture, Forestry and Fisheries (2016). Cha o meguru jousei (The current situation of tea in Japan ) 2016/05ver. Available from: http://www.maff.go.jp/j/seisan/okusan/cha/pdf/cha_meguji_h2805.pdf Accessed on 17, August, 2017 [Online] in Japanese.

Ministry of Health, Labour and Welfare (2016). National health and nutrition survey in Japan 2014, Tokyo in Japanese.

Naito M, Eguchi H, Okada R, et al (2008). Controls for monitoring the deterioration of stored blood samples in the Japan Multi-Institutional Collaborative Cohort Study.
Actual and Estimated Catechins Intakes

(J-MICC Study). *Nagoya J Med Sci*, 70, 107-15.

Otaki N, Kimira M, Katsumata S, et al (2009). Distribution and major sources of flavonoid intakes in the middle-aged Japanese women. *J Clin Biochem Nutr*, 44, 231-8.

Saito E, Inoue M, Sawada N, et al (2015). Association of green tea consumption with mortality due to all causes and major causes of death in a Japanese population: the Japan public health center-based prospective study (JPHC Study). *Ann Epidemiol*, 25, 512-8.

Sasazuki S, Inoue M, Hanaoka T, et al (2004). Green tea consumption and subsequent risk of gastric cancer by subsite: the JPHC Study. *Cancer Causes Control*, 15, 483-91.

Tokudome Y, Goto C, Imaeda N, et al (2005). Relative validity of a short food frequency questionnaire for assessing nutrient intake versus three-day weighed diet records in middle-aged Japanese. *J Epidemiol*, 15, 135-45.

Wu S, Li F, Huang X, et al (2013). The association of tea consumption with bladder cancer risk: a meta-analysis. *Asia Pac J Clin Nutr*, 22, 128-37.

Yu Y, Deng Y, Lu BM, et al (2014). Green tea catechins: a fresh flavor to anticancer therapy. *Apoptosis*, 19, 1-18.

Zhu BH, Zhan WH, Li ZR, et al (2007). (-)-Epigallocatechin-3-gallate inhibits growth of gastric cancer by reducing VEGF production and angiogenesis. *World J Gastroenterol*, 13, 1162-9.