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Plasma tea catechins and risk of cardiovascular disease in middle-aged Japanese subjects: the JPHC Study

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ABSTRACT

Background and Aims: Although a potential benefit of drinking green tea has been suggested to reduce the development of cardiovascular disease, no study has investigated the relationship between plasma tea catechin and risk of cardiovascular disease.

Methods: A prospective, nested case-control study was conducted to examine the association between plasma tea catechin and risk of stroke and coronary heart disease (CHD) in a cohort of 29,876 men and women aged 40–69 years without history of heart disease, stroke or cancer. Participants completed a survey and donated blood samples between 1990 and 1994, and followed-up through 2008. A total of 1,132 stroke cases and 209 CHD cases, matched 1:1 to controls (n=1,132) for stroke and 1:2 to controls (n=418) for CHD, were included in the analysis.

Results: We found no significant association between plasma tea catechin and the incidence of stroke or CHD in either men or women. However, we found that high plasma levels of epigallocatechin gallate (EGCG) were associated with reduced risk of stroke in non-smoking men; the adjusted odds ratio (95% CI) for the highest vs. non-detectable levels was 0.53 (0.29–0.98). The respective OR in male smokers was 1.23 (0.75–2.16). A significant interaction by smoking status was found for the highest vs. non-detected plasma EGCG in relation to stroke (p-for-interaction: p=0.09).

Conclusions: Plasma tea catechin was not associated with reduced risks of either stroke or CHD, while a protective effect of certain tea catechin on stroke risk is suggested for male non-smokers.

Introduction

Because of its high content of catechins (epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCG)), tea consumption has been suggested to protect against the development of cardiovascular disease (CVD) [1].

Several large-scale epidemiological studies have examined the relationship between tea consumption and CVD [2-4]. With regard to stroke, a pooled meta-analysis reported that tea drinking, without any confined effect to black or green tea; was associated with reduced mortality and morbidity of stroke [5]. Indeed, subjects drinking three or more cups of tea per day had a 21% reduced risk of fatal or non-fatal stroke events.[5] Our prospective cohort study of Japanese subjects [6] showed that drinking four or more cups of green tea per day was associated with a 20% lower risk of stroke over 13 years of follow-up. Nevertheless, these studies assessed green tea intake based on a self-administered questionnaire or in-person interview [5, 6]. No study has directly measured pre-diagnostic biomarkers of tea catechins, or examined the risk of stroke in relation to plasma tea catechin levels.

The mechanisms underlying the association between tea catechins and risk of CVD have not been well elucidated, although plausible explanations include the inhibition of oxidative stress and an anti-inflammatory response via antioxidant and free radical scavenging properties [7] which in turn may inhibit hydrogen peroxide, lipid peroxidation, and apoptotic cells [8, 9]. This may result in improved endothelial function [10], inhibition of platelet aggregation [11], and induction of blood vessel relaxation, which lowers blood pressure, and prevents overgrowth of smooth muscle cells in blood vessel walls [12], which can otherwise narrow the blood vessel, reducing blood flow and increasing blood pressure levels. Therefore, considering that anti-

inflammatory catechins represent 80–90% of the total catechins in green tea [13, 14], it is reasonable to assume that green tea would have a beneficial effect on the cardiovascular system.

The catechin content of green tea varies by preparation, type and amount of green tea leaves, water temperature, brewing time, and other factors. To reduce misclassification because of the above variables as well as to further understand the role of tea catechins in the etiology of CVD, it is worth examining this association using pre-diagnostic biomarkers of tea catechins. Therefore, we evaluated the association between levels of plasma tea catechins and the risk of stroke and coronary heart disease (CHD) in a large nested-case control study among approximately 30,000 subjects who provided blood samples during a large prospective cohort of the Japan Public Health Center-based Prospective Study (JPHC Study).

MATERIALS AND METHODS

Study population

The first cohort of the Japan Public Health Center-based Prospective Study (JPHC Study) was initiated in 1990 (Cohort I) and the second in 1993 (Cohort-II) in 11 public health center areas throughout Japan [15]. Two public health center areas (Tokyo and Osaka) were excluded from the present study because data on the incidence of CVD were not available. The study population of the present study was defined as all residents (n = 116,896) aged 40–59 years for Cohort I and 40–69 years for Cohort II at baseline. Of these, 220 were excluded because of non-Japanese nationality (n = 51), late report of emigration occurring before the start of the follow-up period (n = 166), and incorrect birth date (n = 3), leaving 116,676 residents eligible for the study. The present study was approved by the institutional review boards of the National Cancer Center, and Osaka University.

Baseline questionnaire survey and blood collection

A baseline self-administered questionnaire on various aspects of lifestyle was presented to participants in 1990 for Cohort I and in 1993 and 1994 for Cohort II**.** The overall response rate was 82%, giving 95,374 respondents who were included in the study cohort. The questionnaire covered personal and family medical history; psychosocial factors, such as perceived mental stress; household structure; occupation; behavioral patterns; and lifestyle factors, such as smoking and alcohol habits, dietary habits, and physical activity.

Among the study subjects, 34,085 residents donated 10 mL samples of venous blood. Samples were collected into vacutainer tubes containing heparin at the time of

health examinations conducted by the respective municipal governments during the same year as the baseline survey. They were divided into plasma and buffy layers and stored at −80°C until analysis. Of all blood samples, 41% were obtained during fasting i.e. 8 hr or more since the last meal. The mean time difference between the baseline survey and blood sample collection was 9.6 months.

Confirmation of stroke and coronary heart disease

We followed study subjects until December 31, 2007. Those who had died or moved to other municipalities were identified annually through residential registries in their Public Health Center area. Among the study subjects, 9.9% moved away and 0.2% were lost to follow-up during the study period.

A total of 81 hospitals were registered within the administrative districts of the JPHC cohort. All are major hospitals with the capability of treating patients with stroke and acute myocardial infarction (MI). Physicians blinded to patient lifestyle data reviewed medical records at each hospital. Stroke and acute MI events were included in the study if they firstly occurred after the date of return of the baseline questionnaire and before January 1, 2008.

Stroke was confirmed according to the criteria of the National Survey of Stroke [16], which requires the presence of focal neurological deficits of sudden or rapid onset lasting at least 24 hours or until death. Strokes were further classified as hemorrhagic stroke (subarachnoid or intraparenchymal), or ischemic stroke (thrombotic or embolic). All registered hospitals were equipped with computer tomographic scans and/or magnetic resonance scans. A definite diagnosis was established mainly based on an examination of computer tomographic or magnetic resonance images and/or autopsy.

MI was confirmed in the medical records in accordance with the criteria of the MONICA (Monitoring Trends and Determinants of Cardiovascular Disease) project [17], which requires evidence in the form of electrocardiograms, cardiac enzymes, and/or autopsy. In the absence of a diagnosis of MI, deaths that occurred within 1 hour from the onset of symptoms were regarded as sudden cardiac deaths. MI and sudden cardiac deaths were regarded as coronary heart disease.

Selection of case and control subjects

During the study period, a total of 1,132 strokes and 209 CHD occurred among the 29,876 subjects who had returned the baseline questionnaire, reported no prior history of MI, angina pectoris, stroke, or cancer, and provided blood samples.

According to a power calculation, when the percentage of persons with an exposure factor in the control group is set to 0.75, 212 (case/control ratio = 1), 159 (case/control ratio = 2) cases are needed to have a power of 80%, with alpha 5%, to detect an odds ratio of 2.0. For a sufficient number of samples, one control case for each case of stroke and two for each case of CHD were selected using incidence-density sampling [18]. Controls were matched with cases by sex, age (within 2 years), date of blood sampling (within 3 months), time since last meal (within 4 hr) and study location (Public Health Center area). Thus, 1,132 cases and matched 1,132 controls were included in the analysis for stroke, and 209 cases and matched 418 controls were included in the analysis for CHD.

Laboratory assays

Plasma levels of EC, ECG, EGC and EGCG were analyzed using high-performance liquid chromatography with a coulometric array detector in accordance with the

modified methods of Lee et al [19, 20]. Plasma concentrations of tea catechins were determined by linear regression of the peak height for each standard, and adjusted according to the recovery rate of the internal plasma standard. The regression coefficient of peak height and concentration calculated for tea catechins revealed a linearity range 0–0.5 μg/mL, with correlation coefficient values >0.998. The voltammetric response for the standard solution displayed coefficients of variation of 10% for intra- and 11% for inter-day variation. Recovery rates of tea catechins in plasma samples ranged between approximately 63 and 90% (EC, 89%; ECG, 63%; EGC, 90%; and EGCG, 66%). In the present study, tea catechins were measured in two different years (i.e., 2004 and 2008). The minimum detected values are 0.008 μg/mL for EC, 0.002 μg/mL for ECG, 0.004 μg/mL for EGC, and 0.009 μg/mL for EGCG for 2004 and those for 2008 are 0.0006, 0.001, 0.001, and 0.0007 μg/mL, respectively. All analyses of tea catechins were performed at the National Cancer Center, Japan, as described previously [21]. High-sensitivity C-reactive protein (hs-CRP) levels were measured using an ultra-sensitive latex-enhanced immunoassay with an automatic analyzer (BN Prospec nephlomete, Dade Behring, Newark, DE, USA) at Kotobiken Medical Laboratory, Tsukuba, and Osaka Medical Center for Health Science and Promotion. All laboratory personnel were blinded with respect to case or control status.

Statistical analysis

The Wilcoxon–Mann Whitney test was used to compare mean values of baseline cardiovascular risk factors with non-normally distributed values, and chi-square test was used to compare proportions.

Conditional odds ratios (OR) and 95% confidence intervals (95% CI) of stroke and CHD were estimated according to categories for tea catechins using a conditional logistic regression model. The proportions of subjects with detectable levels of EGC, EGCG, ECG and EC were 43%, 40%, 33%, and 42%, respectively (shown in a supplemental figure1). Since the minimum detected values of tea catechins were different at the two measured time points (at the years 2004 and 2008), the detected values of each individual catechin and of total catechin (EGC, EGCG, ECG, plus EC) divided by the tertile of the stroke controls and the median of the CHD controls at each measured time point, used a non-detected level as a reference category. We only adjusted for covariates that were associated with stroke or CHD at a significance level of $p<0.05$ in our study sample, including systolic blood pressure (mmHg), anti-hypertensive medication use (yes *vs.* no), body mass index (BMI; kg/m^2), alcohol intake (non-current drinker, occasional drinker, current drinker), smoking status (current smoker, past smoker, never smoker), diabetic category (normal, borderline or diabetes mellitus), and quartiles of hs-CRP (mg/L). Borderline diabetes mellitus was defined as a fasting glucose level of 6.1–6.9 mmol/L or non-fasting level of 7.8–11.0 mmol/L. Diabetes mellitus was defined as a fasting glucose level of >7.0 mmol/L, a non-fasting level of >11.1 mmol/L, or the use of medication for diabetes.

To check for effect modification, we also stratified analyses according to smoking status (current smoker and non-smoker). All analyses were conducted using the SAS statistical package version 9.1 (SAS Institute Inc., Cary, NC). Statistical differences were considered statistically significant if p values were <0.05 (two-tailed significance levels).

RESULTS

Table 1 shows the sex-specific risk characteristics of stroke and CHD cases and their controls. For men, the average age at the baseline survey from 1990 to 1994 was 56-57 years for both stroke and CHD cases and controls. The prevalence of current smoking, anti-hypertensive medication use, and diabetes mellitus was higher for both stroke and CHD cases than their controls while the prevalence of heavy drinking was lower for CHD cases than for controls. Mean values of systolic blood pressure were also higher for stroke and CHD cases than their controls. Mean values of BMI, total cholesterol and hs-CRP was higher for CHD cases than controls.

For women, the average age at the baseline was 57-59 years for both stroke and CHD cases and controls. The prevalence of anti-hypertensive medication use was higher for both stroke and CHD cases than their controls while the prevalence of current smoking, diabetes mellitus was higher only for stroke cases than for controls. Mean values of systolic blood pressure were also higher for stroke and CHD cases than their controls. Mean values of total cholesterol was higher for stroke cases than for controls, while hs-CRP was higher for CHD cases than controls.

The correlation coefficients between the plasma tea catechin levels and green tea intake are shown in Table 2, and range between 0.14 for ECG and 0.36 for EGC.

Table 3 shows age, sex, date of blood sampling, and time since last meal-matched and multivariable-adjusted conditional odds ratio (95% CI) for stroke and CHD according to the levels of plasma tea catechins (i.e., EGC, EGCG, ECG, EC, and total catechin). We found no significant trends between plasma tea catechins and the incidence of stroke or CHD in either men or women.

We next checked for potential effect modification by stratifying the analyses by smoking status (being and not being a current smoker). Compared with the non-detected plasma EGCG levels, the highest plasma levels of EGCG were associated with a reduced risk of stroke in male non-smokers; the adjusted OR (95% CI) was 0.53 (0.29–0.98) (Table 4). The respective OR in male smokers was 1.23 (0.75–2.16). A significant interaction by smoking status was found for the highest vs. non-detected plasma EGCG levels in relation to stroke (p for interaction: p=0.09).

DISCUSSION

In this population-based prospective nested-case control study in Japanese subjects, we found no associations between the levels of plasma tea catechins and risk of stroke and CHD. However, we found a significant interaction between smoking status, the association between plasma EGCG and the risk of stroke in men. Male non-smokers with the highest levels of plasma EGCG compared with those with non-detectable levels had a reduced risk of stroke, while no association was found in male smokers.

Tea catechins in plasma are partially varied by individual absorption and metabolism, and further differ because of individual tea drinking styles. Of the catechins in a cup of green tea prepared using 1 g of tea leaves brewed for 3 minutes in 100 mL of hot water, EGCG is known to be the most abundant extracted catechin (48–55%) followed by EGC (9–12%), ECG (9–12%) and EC (5–7%) [13, 22]; however, plasma EGCG concentrations account for only 2% of the ingested amount in healthy people [23]. In the present study, the correlation coefficient between the plasma levels of EGCG and green tea intake was low (r = 0.21–0.28 for men and r = 0.25–0.28 for women). However, catechins are widely distributed in fruits, vegetables, and other drinks (i.e., black and oolong tea) [24] and their plasma levels may thus also be affected by these food products. This may help to explain the apparently low correlation between green tea intake and plasma levels of catechin in the present study. Despite their low bioavailability, previous in vivo reports suggest that absorbed catechins may exert beneficial effects [25].

A daily injection of EGCG (200 mg/kg) for 3 weeks improved endothelial function and reduced systolic blood pressure in hypertensive rats [26]. Furthermore, the progression of atherosclerotic plaque formation was reduced in hypercholesterolemic

apolipoprotein E-deficient mice treated with an EGCG (10 mg/kg) daily injection for 6 weeks [27]. A randomized crossover study of 20 healthy male smokers reported a significant improvement of endothelium dependent vasodilatation as well as a decrease in urinary 8-iso prostaglandin F2α, a biomarker of oxidative stress, after consumption of green tea (400 mL) containing 61.8 mg/dL of EGCG [28]. This decrease in urinary 8-iso prostaglandin F2α was also found in seven healthy non-smokers [28]. In the present study, we found a significantly reduced risk of stroke associated with the highest vs. non-detectable plasma levels of EGCG in male non-smokers (despite no association being found for total subjects). EGCG has the strongest anti-oxidative activity among the green tea catechins (EC, ECG, EGC, and EGCG) [29], and its putative beneficial effect on cardiovascular disease is exerted through an oxidative stress-related pathway [30]. However, cigarette smoke exposure increases oxidative stress as a potential mechanism promoting cardiovascular disease [31], and cigarette smoking may thus modify the catechin–cardiovascular disease relationship.

Furthermore, increasing evidence suggests that manipulation of the gut microbiota composition is associated with cardiovascular health [32]. A previous study showed that flavan-3-ol (catechins, proanthocyanidins) modulated the microbiota composition and its catabolic activity, inducing changes that could in turn affect the bioavailability and potential bioactivity of these compounds [33]. The gut microbiota biotransforms flavan-3-ol to bioactive metabolites [34], and these microbe-derived phenolic metabolites have been reported to exert beneficial cardiovascular health effects via antioxidant [35], anti-inflammatory [36], and antithrombotic activities [37]. The conjugated microbial bioconversion products of catechins have been reported to have higher biological

activity than catechins *per se* [34], which might help to explain why the measured catechins were not generally associated with cardiovascular risk in the present study.

Many of the investigations to date suggest that higher consumption of green and black tea does prevent the development of CVD [6, 38-40], despite the null results from some studies [39-40]. However, the present study is the first to provide a more direct test of this hypothesis by looking at the association between plasma tea catechin levels and the risk of CVD under a prospective nested-case control study in an Asian population, in which green tea is a primary drink. We did not find associations between levels of plasma tea catechins and the risk of either stroke or CHD. However, we found some evidence of reduced risks of stroke associated with plasma EGCG among male non-smokers.

The present study has several potential limitations. First, although the study subjects were selected from a general population sample, only 38% of subjects provided blood samples at baseline. According to a previous JPHC report, subjects with and without baseline blood samples differed in socioeconomic status and lifestyle profile; those who provided a sample smoked less, showed greater participation in physical exercise, and had a higher intake of green vegetables and fruits [41]. Given the difficulty of extrapolating risk estimates for our sub-cohort to an entire cohort, any extrapolation of the present results to the general population thus needs to be undertaken with care.

Second, a large percentage of subjects was not having a detectable level of catechins. The plasma levels of tea catechins are affected by time elapsed since the last meal because the plasma half-life is approximately 5 hr for EGCG and approximately 2.5 hr for EGC and EC [42]. We therefore matched fasting time between cases and

controls to minimize the attenuation of risk estimates derived from this measurement error. Furthermore, previous studies found correlations between dietary intake and serum or plasma levels of α- and β-carotene of 0.25–0.58 and 0.15–0.36, respectively [43], indicating that the low bioavailability of tea catechins in the present study may be reasonable.

Third, we measured plasma tea catechins only once for each individual, though blood and urine levels of tea catechins have shown considerable interindividual variation [44-45]. However, green tea consumption levels by most individuals are assumed to be relatively stable over time in Japan, with high reproducibility among repeated measurements of green tea intake by Food Frequency Questionnaire (correlation coefficient = 0.64 for 1-year interval and 0.54 for 5-year interval) (unpublished data).

The strengths of our study are its prospective population-based design and status as the largest nested case-control study of this association, which yielded good statistical power in detecting the effects of tea catechins. Although there were a sufficient number of samples, our findings may be limited because of the small number of CHD cases. Thus, further research is needed to assess the repeatability of the association between the levels of plasma tea catechins and risk of CHD with a larger number of cases.

Ours is the first investigation of plasma tea catechins and risks of stroke and CHD incidences. The present study found no associations between plasma levels of tea catechin and risks of stroke and CHD among the subjects overall. However, plasma EGCG levels were associated with a possible reduced risk of stroke among male non-smokers, suggesting that tea could be a useful component of a healthy diet.

Conflict of interest: None declared.

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Author contributions: Ikeda and Iso had the original idea and developed the study design. Ikeda performed the statistical analyses and wrote the first draft of the manuscript. All authors collected data and contributed to the critical revision of the manuscript. All authors read and approved the final manuscript.

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JPHC members are listed at the following site (as of April 2017);

http://epi.ncc.go.jp/en/jphc/781/7951.html

REFERENCES

1. Riemersma RA, Rice-Evans CA, Tyrrell RM, Clifford MN, Lean ME. Tea flavonoids and cardiovascular health. QJM 2001; 94:277-282.
2. Geleijnse JM, Launer LJ, Van der Kuip DA, Hofman A, Witteman JC. Inverse association of tea and flavonoid intakes with incident myocardial infarction: the Rotterdam Study. Am J Clin Nutr. 2002; 75:880-886.
3. Kuriyama S, Shimazu T, Ohmori K, Kikuchi N, Nakaya N, Nishino Y, et al. Green tea consumption and mortality due to cardiovascular disease, cancer, and all causes in Japan: the Ohsaki study. JAMA 2006; 296:1255-1265.
4. de Koning Gans JM, Uiterwaal CS, van der Schouw YT, Boer JM, Grobbee DE, Verschuren WM, et al. Tea and coffee consumption and cardiovascular morbidity and mortality. Arterioscler Thromb Vasc Biol 2010; 30:1665-1671.
5. Arab L, Liu W, Elashoff D. Green and black tea consumption and risk of stroke: a meta-analysis. Stroke 2009; 40: 1786-1792.
6. Kokubo Y, Iso H, Saito I, Yamagishi K, Yatsuya H, Ishihara J, et al. The impact of green tea and coffee consumption on the reduced risk of stroke incidence in Japanese population: the Japan public health center-based study cohort. Stroke 2013; 44: 1369-1374.
7. Sutherland BA, Rahman RM, Appleton I. Mechanisms of action of green tea catechins, with a focus on ischemia-induced neurodegeneration. J Nutr Biochem 2006; 17: 291-306.
8. Hong JT, Ryu SR, Kim HJ, Lee JK, Lee SH, Kim DB, et al. Neuroprotective effect of green tea extract in experimental ischemia-reperfusion brain injury. Brain Res Bull 2000; 53: 743-749.

9. Hong JT, Ryu SR, Kim HJ, Lee JK, Lee SH, Yun YP, et al. Protective effect of green tea extract on ischemia/reperfusion-induced brain injury in Mongolian gerbils. Brain Res 2001; 888:11-18.
10. Ludwig A, Lorenz M, Grimbo N, Steinle F, Meiners S, Bartsch C, et al. The tea flavonoid epigallocatechin-3-gallate reduces cytokine-induced VCAM-1 expression and monocyte adhesion to endothelial cells. Biochem Biophys Res Commun 2004; 316: 659-665.
11. Neiva TJ, Morais L, Polack M, Simões CM, D'Amico EA. Effects of catechins on human blood platelet aggregation and lipid peroxidation. Phytother Res 1999; 13: 597-600.
12. Soriani M, Rice-Evans C, Tyrrell RM. Modulation of the UVA activation of haem oxygenase, collagenase and cyclooxygenase gene expression by epigallocatechin in human skin cells. FEBS Lett 1998; 439: 253-257.
13. Balentine DA, Wiseman SA, Bouwens LC. The chemistry of tea flavonoids. Crit Rev Food Sci Nutr 1997; 37: 693-704.
14. Babu PV, Liu D. Green tea catechins and cardiovascular health: an update. Curr Med Chem 2008; 15: 1840-1850.
15. Tsugane S, Sawada N. The JPHC study: design and some findings on the typical Japanese diet. Jpn J Clin Oncol 2014; 44:777–82.
16. Walker AE, Robins M, Weinfeld FD. The National Survey of Stroke. Clinical findings. Stroke 1981; 12: I13-I44.
17. Tunstall-Pedoe H, Kuulasmaa K, Amouyel P, Arveiler D, Rajakangas AM, Pajak A. Myocardial infarction and coronary deaths in the World Health Organization

MONICA Project. Registration procedures, event rates, and case-fatality rates in 38 populations from 21 countries in four continents. Circulation 1994; 90: 583-612.

18. Rothman KJ, Greenland S. Measures of effect and association. In: Modern Epidemiology. Philadelphia, Pa: Lippincott Williams & Wilkins;1998:47–64.
19. Lee MJ, Prabhu S, Meng X, Li C, Yang CS. An improved method for the determination of green and black tea polyphenols in biomatrices by high-performance liquid chromatography with coulometric array detection. Anal Biochem 2000; 279: 164-169.
20. Lee MJ, Wang ZY, Li H, Chen L, Sun Y, Gobbo S, et al. Analysis of plasma and urinary tea polyphenols in human subjects. Cancer Epidemiol Biomarkers Prev 1995; 4: 393-399.
21. Iwasaki M, Inoue M, Sasazuki S, Miura T, Sawada N, Yamaji T, et al. Plasma tea polyphenol levels and subsequent risk of breast cancer among Japanese women: a nested case-control study. Breast Cancer Res Treat 2010; 124: 827-834.
22. Shahidi F. Antioxidants in food and food antioxidants. Nahrung 2000; 44:158-163.
23. Nakagawa K, Okuda S, Miyazawa T. Dose-dependent incorporation of tea catechins, (-)-epigallocatechin-3-gallate and (-)-epigallocatechin, into human plasma. Biosci Biotechnol Biochem 1997; 61: 1981-1985.
24. Nutrient Data Laboratory US Department of Agriculture, USDA Database for the Flavonoid Content of Selected Foods. US Department of Agriculture, Agricultural Research Service, 2007.
25. Basu A, Lucas EA. Mechanisms and effects of green tea on cardiovascular health. Nutr Rev 2007; 65: 361-375.

26. Potenza MA, Marasciulo FL, Tarquinio M, Tiravanti E, Colantuono G, Federici A, et al. EGCG, a green tea polyphenol, improves endothelial function and insulin sensitivity, reduces blood pressure, and protects against myocardial I/R injury in SHR. Am J Physiol Endocrinol Metab 2007; 292: E1378-1387.

27. Chyu KY, Babbidge SM, Zhao X, Dandillaya R, Rietveld AG, Yano J, et al. Differential effects of green tea-derived catechin on developing versus established atherosclerosis in apolipoprotein E-null mice. Circulation 2004; 109: 2448-2453.

28. Nagaya N, Yamamoto H, Uematsu M, Itoh T, Nakagawa K, Miyazawa T, et al. Green tea reverses endothelial dysfunction in healthy smokers. Heart 2004; 90: 1485-1486.

29. Ishikawa T, Suzukawa M, Ito T, Yoshida H, Ayaori M, Nishiwaki M, et al. Effect of tea flavonoid supplementation on the susceptibility of low-density lipoprotein to oxidative modification. Am J Clin Nutr 1997; 66: 261-266.

30. Griffiths K, Aggarwal BB, Singh RB, Buttar HS, Wilson D, De Meester F. Food Antioxidants and Their Anti-Inflammatory Properties: A Potential Role in Cardiovascular Diseases and Cancer Prevention. Diseases 2016; 1: 4(3).

31. Ambrose JA, Barua RS. The pathophysiology of cigarette smoking and cardiovascular disease: an update. J Am Coll Cardiol 2004; 43: 1731-1737.

32. Tang WH, Kitai T, Hazen SL. Gut Microbiota in Cardiovascular Health and Disease. Circ Res 2017; 120: 1183-1196.

33. Cueva C1, Sánchez-Patán F, Monagas M, Walton GE, Gibson GR, Martín-Álvarez PJ, et al. In vitro fermentation of grape seed flavan-3-ol fractions by human faecal microbiota: changes in microbial groups and phenolic metabolites. FEMS Microbiol Ecol 2013; 83: 792-805.

34. Monagas M, Urpi-Sarda M, Sánchez-Patán F, Llorach R, Garrido I, Gómez-Cordovés C, et al. Insights into the metabolism and microbial biotransformation of dietary flavan-3-ols and the bioactivity of their metabolites. Food Funct 2010; 1: 233–253.
35. Grimm T, Schäfer A, Högger P. Antioxidant activity and inhibition of matrix metalloproteinases by metabolites of maritime pine bark extract (Pycnogenol). Free Radic Biol Med 2004; 36: 811–822.
36. Larrosa M, Luceri C, Vivoli E, Pagliuca C, Lodovici M, Moneti G, et al. Polyphenol metabolites from colonic microbiota exert anti-inflammatory activity on different inflammation models. Mol Nutr Food Res 2009; 53: 1044–1054.
37. Rechner AR, Kroner C. Anthocyanins and colonic metabolites of dietary polyphenols inhibit platelet function. Thromb Res 2005; 116: 327–334.
38. Arab L, Liu W, Elashoff D. Green and black tea consumption and risk of stroke: a meta-analysis. Stroke 2009; 40: 1786-1792.
39. Wang ZM, Zhou B, Wang YS, Gong QY, Wang QM, Yan JJ, et al. Black and green tea consumption and the risk of coronary artery disease: a meta-analysis. Am J Clin Nutr 2011; 93: 506-515.
40. Deka A, Vita JA. Tea and cardiovascular disease. Pharmacol Res 2011; 64: 136-145.
41. Iwasaki M, Yamamoto S, Otani T, Inoue M, Hanaoka T, Sobue T, et al. Generalizability of relative risk estimates from a well-defined population to a general population. Eur J Epidemiol 2006; 21: 253-262.
42. Lee MJ, Maliakal P, Chen L, Meng X, Bondoc FY, Prabhu S, et al. Pharmacokinetics of tea catechins after ingestion of green tea and (-)-

epigallocatechin-3-gallate by humans: formation of different metabolites and individual variability. Cancer Epidemiol Biomarkers Prev 2002; 11: 1025-1032.

43. Kobayashi M, Sasaki S, Tsugane S. Validity of a self-administered food frequency questionnaire used in the 5-year follow-up survey of the JPHC Study Cohort I to assess carotenoids and vitamin C intake: comparison with dietary records and blood level. J Epidemiol 2003; 13: S82 – 91.
44. Yang CS, Chen L, Lee MJ, Balentine D, Kuo MC, Schantz SP. Blood and urine levels of tea catechins after ingestion of different amounts of green tea by human volunteers. Cancer Epidemiol Biomarkers Prev 1998; 7: 351 – 354.
45. Chow HH, Cai Y, Alberts DS, Hakim I, Dorr R, Shahi F, et al. Phase I pharmacokinetic study of tea polyphenols following single-dose administration of epigallocatechin gallate and polyphenon E. Cancer Epidemiol Biomarkers Prev 2001; 10: 53-58.

Table 1. Characteristics of case and matched control subjects at baseline

	Men			Women		
	Stroke case n=583	Stroke control n=583	*P* for difference	Stroke case n=549	Stroke control n=549	*P* for difference
Age, year	57.4	57.3	0.80	57.2	57.1	0.82
Body mass index, kg/m^2	23.7	23.5	0.18	24.6	24.0	0.003
Current smoker,%	50.0	40.3	<0.001	5.85	2.37	0.004
Heavy alcohol intake (≥450mg/week),%	71.8	65.9	0.04	7.22	7.92	0.67
Systolic blood pressure, mmHg	141.7	134.1	<0.001	139.0	132.1	<0.001
Antihypertesive drug use,%	26.4	14.9	<0.001	31.3	17.3	<0.001
Diabetes mellitus,%	11.9	5.14	<0.001	8.77	4.48	0.01
Serum total cholesterol levels, mmol	194.6	193.3	0.54	209.6	205.1	0.04
hs-CRP, mg/l	1.74	1.22	0.08	1.16	1.11	0.73
Green tea intake, g/day	440.1	457.5	0.39	402.8	399.7	0.87
Chinese tea intake, g/day	35.5	40.7	0.49	58.1	53.3	0.63
Black tea intake, g/day	11.6	9.7	0.53	11.3	11.3	1.00
Fruit juice intake, g/day	23.5	26.7	0.33	27.1	24.7	0.53
Vegetable juice intake, g/day	14.6	12.5	0.45	13.9	15.7	0.62
Vegetable intake, g/day	114.3	113.6	0.90	127.0	131.6	0.46
Green and yellow vegetable, g/day	29.9	30.5	0.62	38.3	38.0	0.83
Fruit intake, g/day	73.3	79.7	0.15	109.3	109.8	0.93

Hs-CRP: high-sensitivity C-reactive protein

Table 2. Spearman correlation coefficients between green tea consumption (g/week) and ordinal categories of plasma tea catechin[a]

Stroke	**Men**			**Women**	
	r	*p*--value		r	*p*--value
EGC	0.36	<0.001	EGC	0.34	<0.001
EC	0.27	<0.001	EC	0.27	<0.001
EGCG	0.28	<0.001	EGCG	0.28	<0.001
ECG	0.27	<0.001	ECG	0.24	<0.001
Total catechin	0.31	<0.001	Total catechin	0.31	<0.001
CHD					
EGC	0.23	<0.001	EGC	0.30	<0.001
EC	0.24	<0.001	EC	0.22	<0.001
EGCG	0.21	<0.001	EGCG	0.25	<0.001
ECG	0.17	<0.001	ECG	0.14	0.04
Total catechin	0.20	<0.001	Total catechin	0.27	<0.001

[a]Categories of plasma tea catechin were defined as 0 for non-detected; 1 through 3 for detected tea catechins divided into tertiles for stroke and 1 through 2 for detected tea catechins divided into median for coronary heart disease (CHD).

EGC: epigallocatechin, EC: epicatechin, EGCG: epigallocatechin-3-gallate, ECG: epicatechin-3-gallate

Table 3. Odds ratios (ORs) and 95% confidence intervals (CIs) of stroke and CHD according to plasma tea catechin levels

Stroke	**Men**					**Women**				
	Tertile					**Tertile**				
	Non-detected	**T1**	**T2**	**T3**	***P* for trend**	**Non-detected**	**T1**	**T2**	**T3**	***P* for Trend**
EGC (ng/ml)										
Median [a]	0.0000	0.0273	0.1498	0.5773		0.0000	0.0301	0.1198	0.4762	
Median [b]	0.0000	0.0019	0.0080	0.0352		0.0000	0.0022	0.0077	0.0239	
No. of cases/no. of controls	315/334	96/82	90/84	82/83		331/341	72/69	81/70	65/69	
OR (95% CI) [c]	1.00	1.26(0.90-1.77)	1.15(0.81-1.63)	1.05(0.70-1.57)	0.54	1.00	1.08(0.73-1.60)	1.20(0.82-1.75)	0.96(0.63-1.48)	0.77
Multivariate OR (95% CI) [d]	1.00	1.32(0.91-1.93)	0.99(0.67-1.46)	0.97(0.62-1.51)	1.00	1.00	1.04(0.68-1.60)	1.13(0.75-1.72)	0.95(0.58-1.54)	0.91
EC (ng/ml)										
Median [a]	0.0000	0.0208	0.0563	0.1702		0.0000	0.0168	0.0578	0.1158	
Median [b]	0.0000	0.0009	0.0023	0.0111		0.0000	0.0009	0.0021	0.0062	
No. of cases/no. of controls	324/353	91/76	84/78	84/76		331/338	70/69	76/71	72/71	
OR (95% CI) [c]	1.00	1.34(0.94-1.91)	1.25(0.86-1.83)	1.29(0.86-1.94)	0.12	1.00	1.05(0.70-1.56)	1.10(0.75-1.61)	1.05(0.70-1.57)	0.69
Multivariate OR (95% CI) [d]	1.00	1.18(0.80-1.75)	1.13(0.74-1.74)	1.15(0.74-1.79)	0.44	1.00	0.98(0.63-1.52)	0.99(0.65-1.50)	1.02(0.65-1.59)	0.97
EGCG (ng/ml)										
Median [a]	0.0000	0.0281	0.0668	0.1974		0.0000	0.0253	0.0603	0.1434	
Median [b]	0.0000	0.0012	0.0027	0.0114		0.0000	0.0011	0.0025	0.0062	
No. of cases/no. of controls	320/345	108/79	79/80	76/79		337/344	75/68	73/69	64/68	
OR (95% CI) [c]	1.00	1.56(1.10-2.21)	1.10(0.76-1.60)	1.04(0.70-1.54)	0.66	1.00	1.13(0.79-1.63)	1.07(0.70-1.64)	0.97(0.64-1.45)	0.98
Multivariate OR (95% CI) [d]	1.00	1.56(1.06-2.29)	0.97(0.64-1.47)	0.83(0.53-1.29)	0.56	1.00	1.07(0.72-1.60)	1.01(0.63-1.62)	0.84(0.53-1.32)	0.57

ECG (ng/ml)										
Median [a]	0.0000	0.0076	0.0156	0.0359		0.0000	0.0080	0.0161	0.0304	
Median [b]	0.0000	0.0015	0.0024	0.0059		0.0000	0.0016	0.0028	0.0059	
No. of cases/no. of controls	379/390	72/64	81/65	51/64		391/405	64/48	38/48	56/48	
OR (95% CI) [c]	1.00	1.19(0.81-1.74)	1.25(0.85-1.84)	0.78(0.49-1.25)	0.96	1.00	1.39(0.92-2.12)	0.82(0.49-1.38)	1.28(0.76-2.14)	0.43
Multivariate OR (95% CI) [d]	1.00	1.21(0.80-1.84)	1.19(0.77-1.84)	0.70(0.42-1.17)	0.60	1.00	1.16(0.73-1.83)	0.84(0.47-1.50)	1.34(0.75-2.39)	0.47
Total catechin (ng/ml)										
Median [a]	0.0000	0.0365	0.2336	0.8745		0.0000	0.0339	0.1696	0.6763	
Median [b]	0.0000	0.0016	0.0071	0.0415		0.0000	0.0015	0.0061	0.0282	
No. of cases/no. of controls	243/261	106/107	118/108	116/107		251/260	101/95	107/97	90/97	
OR (95% CI) [c]	1.00	1.09(0.77-1.54)	1.23(0.87-1.73)	1.23(0.84-1.80)	0.42	1.00	1.13(0.80-1.60)	1.17(0.81-1.68)	0.94(0.62-1.43)	0.51
Multivariate OR (95% CI) [d]	1.00	0.98(0.67-1.44)	1.11(0.76-1.61)	1.09(0.72-1.66)	0.72	1.00	1.08(0.73-1.58)	1.09(0.73-1.63)	0.87(0.55-1.40)	0.42

CHD	**Median**				**Median**			
	Non-detected	**M1**	**M2**	***P* for trend**	**Non-detected**	**M1**	**M2**	***P* for trend**
EGC (ng/ml)								
Median [a]	0.0000	0.0728	0.5686		0.0000	0.0471	0.3905	
Median [b]	0.0000	0.0141	0.1996		0.0000	0.0127	0.2919	
No. of cases/no. of controls	64/133	42/66	27/67		40/92	15/30	21/30	
OR (95% CI) [c]	1.00	1.35(0.76-2.37)	0.85(0.46-1.60)	0.67	1.00	1.34(0.60-3.00)	2.20(0.93-5.22)	0.08
Multivariate OR (95% CI) [d]	1.00	0.97(0.47-2.00)	0.78(0.35-1.74)	0.55	1.00	1.29(0.52-3.16)	1.99(0.70-5.71)	0.20

EC (ng/ml)								
Median [a]	0.0000	0.0224	0.1304		0.0000	0.0198	0.0723	
Median [b]	0.0000	0.0083	0.0467		0.0000	0.0084	0.0512	
No. of cases/no. of controls	72/140	35/62	26/64		40/86	17/33	19/33	
OR (95% CI) [c]	1.00	1.07(0.63-1.84)	0.75(0.40-1.38)	0.42	1.00	1.28(0.58-2.84)	1.49(0.63-3.53)	0.35
Multivariate OR (95% CI) [d]	1.00	1.08(0.54-2.13)	0.96(0.43-2.15)	0.97	1.00	1.26(0.53-3.03)	1.20(0.44-3.28)	0.66
EGCG (ng/ml)								
Median [a]	0.0000	0.0388	0.1386		0.0000	0.0262	0.2238	
Median [b]	0.0000	0.0232	0.0679		0.0000	0.0186	0.1034	
No. of cases/no. of controls	81/165	28/50	24/51		50/103	8/24	18/25	
OR (95% CI) [c]	1.00	1.15(0.65-2.06)	0.96(0.52-1.77)	1.00	1.00	0.78(0.32-1.88)	1.71(0.75-3.88)	0.30
Multivariate OR (95% CI) [d]	1.00	0.96(0.46-2.02)	0.95(0.43-2.08)	0.89	1.00	0.55(0.21-1.47)	1.51(0.56-4.05)	0.68
ECG (ng/ml)								
Median [a]	0.0000	0.0119	0.0273		0.0000	0.0057	0.0411	
Median [b]	0.0000	0.0067	0.0161		0.0000	0.0045	0.0166	
No. of cases/no. of controls	76/163	31/51	26/52		43/93	11/28	22/31	
OR (95% CI) [c]	1.00	1.43(0.78-2.64)	1.15(0.63-2.09)	0.58	1.00	0.91(0.35-2.41)	1.81(0.79-4.12)	0.16
Multivariate OR (95% CI) [d]	1.00	0.89(0.39-2.00)	0.87(0.40-1.92)	0.73	1.00	0.68(0.22-2.07)	1.52(0.58-4.02)	0.40
Total catechin (ng/ml)								
Median [a]	0.0000	0.1565	0.8830		0.0000	0.0587	0.3414	
Median [b]	0.0000	0.0141	0.2703		0.0000	0.0142	0.2251	
No. of cases/no. of controls	54/113	44/76	35/77		30/70	22/40	24/42	
OR (95% CI) [c]	1.00	1.27(0.69-2.34)	1.00(0.51-1.93)	0.57	1.00	1.58(0.71-3.55)	2.01(0.80-5.02)	0.26

Multivariate OR (95% CI) [d]	1.00	1.18(0.53-2.65)	1.02(0.43-2.43)	0.79	1.00	1.69(0.70-4.09)	1.55(0.53-4.48)	0.76

[a] Measured at 2004; [b] Measured at 2008; [c] Matched for age, sex, date of blood sampling, time since last meal, and study location; [d] Adjusted for systolic blood pressure, anti-hypertensive medication use, diabetes mellitus, body mass index, alcohol intake, smoking status, and high-sensitivity C-reactive protein.

Table 4. Smoking status specific odds ratios (ORs) and 95% confidence intervals (CIs) of stroke according to plasma tea catechin levels in men.

	Non-smokers					Smokers				
	Tertiles					Tertiles				
	Non-detected	T1	T2	T3	*P* for trend	Non-detected	T1	T2	T3	*P* for trend
EGC (ng/ml)										
Median [a]	0.0000	0.0251	0.1712	0.7290		0.0000	0.0281	0.0865	0.5236	
Median [b]	0.0000	0.0020	0.0084	0.0319		0.0000	0.0017	0.0073	0.0364	
No. of cases/no. of controls	168/207	52/52	46/45	23/39		144/120	43/29	44/39	58/44	
Multivariate OR (95% CI) [c]	1.00	1.15(0.73-1.82)	1.19(0.72-1.97)	0.68(0.37-1.25)	0.62	1.00	1.43(0.81-2.51)	0.86(0.51-1.46)	1.23(0.74-2.04)	0.65
EC (ng/ml)										
Median [a]	0.0000	0.0224	0.0562	0.2246		0.0000	0.0164	0.0576	0.1637	
Median [b]	0.0000	0.0009	0.0021	0.0104		0.0000	0.0009	0.0025	0.0142	
No. of cases/no. of controls	165/220	62/47	37/37	25/39		156/125	28/29	47/41	58/37	
Multivariate OR (95% CI) [c]	1.00	1.62(1.02-2.57)	1.41(0.82-2.43)	0.74(0.40-1.36)	1.00	1.00	0.72(0.39-1.31)	0.88(0.52-1.49)	1.26(0.74-2.15)	0.58
EGCG (ng/ml)										
Median [a]	0.0000	0.0286	0.0799	0.2084		0.0000	0.0277	0.0634	0.1963	
Median [b]	0.0000	0.0010	0.0028	0.0118		0.0000	0.0013	0.0027	0.0083	
No. of cases/no. of controls	168/205	64/54	37/43	20/41		149/133	43/24	42/37	55/38	
Multivariate OR (95% CI) [c]	1.00	1.36(0.87-2.13)	0.96(0.57-1.63)	0.53(0.29-0.98)	0.14	1.00	1.77(0.98-3.20)	0.97(0.56-1.67)	1.28(0.75-2.16)	0.47
ECG (ng/ml)										

Median [a]	0.0000	0.0085	0.0152	0.0402		0.0000	0.0071	0.0157	0.0355	
Median [b]	0.0000	0.0015	0.0024	0.0059		0.0000	0.0015	0.0024	0.0056	
No. of cases/no. of controls	202/238	35/37	35/34	17/34		173/146	37/26	46/30	33/30	
Multivariate OR (95% CI) [c]	1.00	1.03(0.61-1.76)	1.15(0.67-1.98)	0.53(0.27-1.02)	0.26	1.00	1.26(0.70-2.25)	1.22(0.71-2.09)	1.00(0.55-1.79)	0.70
Total catechin (ng/ml)										
Median [a]	0.0000	0.0349	0.2490	1.0747		0.0000	0.0365	0.1730	0.8601	
Median [b]	0.0000	0.0017	0.0064	0.0417		0.0000	0.0016	0.0080	0.0413	
No. of cases/no. of controls	125/156	57/71	69/62	38/54		115/100	48/33	49/46	77/53	
Multivariate OR (95% CI) [c]	1.00	0.92(0.59-1.44)	1.32(0.85-2.05)	0.80(0.48-1.33)	0.34	1.00	1.43(0.83-2.49)	0.91(0.55-1.52)	1.35(0.85-2.15)	0.27

[a] Measured at 2004; [b] Measured at 2008; [c] Adjusted for systolic blood pressure, anti-hypertensive medication use, diabetes mellitus, body mass index, alcohol intake, smoking status, and high-sensitivity C-reactive protein.