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Coffee and Green Tea As a Large Source of Antioxidant Polyphenols in the Japanese Population

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Food and beverages rich in polyphenols with antioxidant activity are highlighted as a potential factor for risk reduction of lifestyle related diseases. This study was conducted to elucidate total polyphenol consumption from beverages in Japanese people. Total polyphenol (TP) contents in beverages were measured using a modified Folin-Ciocalteu method removing the interference of reduced sugars by using reverse-phase column chromatography. A beverage consumption survey was conducted in the Tokyo and Osaka areas in 2004. Randomly selected male and female subjects (10-59 years old, n = 8768) recorded the amounts and types of all nonalcoholic beverages consumed in a week. Concentration of TP in coffee, green tea, black tea, Oolong tea, barley tea, fruit juice, tomato/vegetable juice, and cocoa drinks were at 200, 115, 96, 39, 9, 34, 69, and 62 mg/100 mL, respectively. Total consumption of beverages in a Japanese population was 1.11 ± 0.51 L/day, and TP contents from beverages was 853 \pm 512 mg/day. Coffee and green tea shared 50% and 34% of TP consumption in beverages, respectively, and contribution of each of the other beverages was less than 10%. TP contents in 20 major vegetables and 5 fruits were 0-49 mg and 2-55 mg/100 g, respectively. Antioxidant activities, Cu reducing power, and scavenging activities for DPPH and superoxide, of those samples correlated to the TP contents ($p \le 0.001$). Beverages, especially coffee, contributed to a large share of the consumption of polyphenols, as antioxidants, in the Japanese diet.

KEYWORDS: polyphenol; consumption; beverage; coffee; tea; fruit and vegetable juices; antioxidant

INTRODUCTION

Polyphenols are major compounds diversely existing in plants, especially in their seeds and green leaves, to protect them from oxidative damage through their antioxidant activity (1). Oxidative stress is a key factor for human disease risk, and some foods and beverages rich in polyphenols are highlighted as possibly contributing to reduced risk of several diseases (2). Coffee, rich in chlorogenic acids, a nonflavonoid polyphenol, is one of the most well-documented foods with epidemiological study results. The risk of type 2 diabetes (3-5), liver cirrhosis (6, 7), liver and colorectal cancer (8-10), and risk of death attributed to inflammatory and cardiovascular diseases (11) have been shown

to be reduced by coffee consumption in some people. Cocoa, rich in epicatechin, could suppress LDL oxidation (12), and may reduce the risk of coronary heart diseases (13, 14). Green tea may also contribute to prevention of cancer and mortality from coronary heart diseases (7, 15). Fruits and vegetables containing antioxidative vitamins are also suggested to have a prophylactic effect on cancer (16, 17) and stroke (18). Polyphenols are varied molecules containing approximately 5000 species including flavonoids (e.g., catechins in tea, isoflavons in beans, apigenin, quercetin, and lutein in vegetables, and anthocyanins in fruit) and nonflavonids (chlorogenic acids in coffee). The contents of polyphenols in plants range from a few milligrams to hundreds of milligrams per 100 g of fresh weight, and sometimes reaches more than 100 times higher than that of antioxidative vitamins (19).

Several researchers have reported on the consumption of polyphenols. Svilaas et al. estimated that the daily consumption of antioxidants in Norwegians was 17 mmol (4.9 g as catechin equivalent) of which 65%, 11%, 8%, and 5% was consumed by coffee, fruits, black tea, and wine, respectively (20). Vinson

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showed that a major source of polyphenol in U.S. residents is fruits and vegetables, of which 255 and 218 mg per day are consumed, respectively (21, 22). These two studies measured total polyphenols by the Folin-Ciocalteu method. Hertog et al. reported that the Dutch consume 23 mg/day of 5 major flavonoids from fruits, vegetables, and beverages based on a national food consumption survey and measurement of flavonoids using the HPLC method (23).

Measurement of polyphenols using the HPLC method is an accurate way to quantify the amount of each polyphenol molecule. However, it is difficult to measure all polyphenol molecules, which are diversely distributed, by using the limited standard samples, and the assumption therefore can sometimes be underestimated. On the contrary, a colorimetric measurement such as the Folin-Ciocalteu method for polyphenol content can be simply conducted and has advantages to roughly estimate overall amounts of polyphenols, whose molecules are largely diversed. There is difficulty in the Folin-Ciocalteu method to remove the interference of nonpolyphenol compounds such as reduced sugars including vitamin C, and some researchers separately measure vitamin C for adjustment (22). George et al. recently reported a modified Folin-Ciocalteu method using reverse-phase column chromatography to successfully remove the interference by nonpolyphenol compounds including not only vitamin C but also the other chemicals that possibly interfere with the system (24).

Consumption of food and beverage is different in each country and society quantatively and qualitatively. Many studies showed the contents of polyphenols in food and beverages (25, 26), and beverages could be a large source of polyphenols; however, statistics of the consumption data on beverages are limited. In this study, we conducted a survey of beverage consumption and a measurement of total polyphenol contents in beverages and major fruits and vegetables consumed in Japan, aiming at elucidating the polyphenol consumption in the Japanese population.

MATERIALS AND METHODS

Samples. Beverages, vegetables, fruits, red wine, and black pepper were purchased at a supermarket located in Kobe, Japan, in the spring, summer, and autumn seasons. After the samples were purchased, total polyphenol measurement was immediately conducted. Cacao mass was supplied by Fuji Oil Co. (Osaka, Japan).

Materials. L-Ascorbic acid (vitamin C), sodium carbonate (Na₂CO₃), and Trolox were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Folin-Ciocalteu reagent and diethylenetriaminepentaacetic acid (DETAPAC) were purchased from Sigma Aldrich Japan K.K. (Tokyo, Japan). Xanthine oxidase, 5,5-dimethyl-l-pyrroline Noxide (DMPO), and superoxide dismutase (SOD) were purchased from LABTEC Company Ltd. (Kyoto, Japan). AS standards of polyphenols, catechin, epigallocatechin, epigallocatechin gallate, epicatechin gallate, epicatechin, and naringenin, from Funakoshi Co., Ltd., (Tokyo, Japan), tannic acid from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), chlorogenic acid from Tokyo Chemical Industry Co., Ltd., (Tokyo, Japan), and gallic acid ethyl ester from Kanto Chemical Co.,Inc. (Tokyo, Japan) were used.

Total Polyphenol Contents. Total polyphenol (TP) content in beverages was measured using a modified Folin-Ciocalteu method described by George et al. (*24*). Briefly, beverage samples were extracted by acetone/water solution (7:3 (v/v)) and solvent extract (SE) solution was obtained after filtration. An aliquot of SE solution diluted at less than 7% of acetone was applied on an Oasis HLB cartridge (Waters Japan, Tokyo) and absorbed polyphenols, followed by eluted washing extract (WE) solution containing interfering water-soluble components, reducing sugars, and ascorbic acid. A 1/10 water-diluted Folin-Ciolcateu reagent was added to the SE and WE solutions, adding 75 g/L of sodium carbonate, and the solution was incubated for 15

min at 50 °C. Then the specific absorbance at 760 nm was measured on an Hitachi 200-10 model spectrophotometer (UC-160A, Shimadzu Co., (Kyoto, Japan). Total polyphenols were determined by subtracting the SE from that of WE, after calculating milligrams of chlorogenic acid and catechin (Sigma-Aldrich Japan KK, Tokyo) equivalent values for 100 g or mL of coffee and the other beverages, respectively.

Preparation of extracts from fruits and vegetables was conducted as follows: 10 g of a fresh edible portion of fruits and vegetables was chopped and homogenized in an extract solution, 70% ethanol and 0.9% NaCl (7:3), for 1 min with a homogenizer (Nissei Biomixer BM-2, Nihon Seiki Kaisha Ltd., Tokyo, Japan), the solution was allowed to stand in a sonicator for 10 min at 4 °C, and the supernatant was obtained by centrifugation at 3000 rpm for 5 min. Extraction was repeated twice using sonic treatment for 5 min, and stored at 4 °C. The supernatant (RE) was submitted to the Folin-Ciocalteu method described above.

Antioxidant Activities. Cu reducing activity was determined by using the PAO test kit (Nikken ZEIL Co., Ltd., Fukuroi, Japan). Samples were diluted in the buffer supplied with the kit containing Cu^{2+} and placed on 96-well plates, and their absorbance at 490 nm was determined in a micro plate reader (Bio-Rad Laboratories K.K., Tokyo, Japan) to obtain blank values. Bathocuproine solution was added and incubated at room temperature for 3 min and then measured for absorbance at 490 nm. A standard curve was prepared using uric acid.

DPPH radical scavenging activity of sample extracts was determined using an electron-spin resonance (ESR) method. A sample extract (100 μ L) was mixed with 100 μ L of 48 μ M DPPH solution diluted with ethanol and applied to an ESR spectrometer (model JES-FR80. JEOL Ltd. (Tokyo, Japan), the ESR value measured (frequency: 9.415 GHz; power: 10 mW; center field: 335 ± 5 mT; field modulation frequency: 100 kHz; field modulation width: 0.79 × 0.1 mT; receiver gain: 3.2 × 100; time constant: 0.3 s; sweep time: 2 min) at room temperature after a 1 min reaction. Trolox with 50% methanol S was used as a standard.

Super oxide radical scavenging activity (SOSA) of beverage samples was determined by the hypoxanthine-xanthine oxidase superoxide generating system. Fifty microliters of hypoxanthine was placed in a test tube, and 40 μ L of DETAPAC (5.5 μ M), 50 μ L of distilled water, 10 μ L of DMPO, and 50 μ L of xanthine oxidase were added successively. The assay was carried out on an ESR spectrometer after 40 s of incubation at 20 °C. The relative intensity (RI) ratio of the superoxide signal intensity to the manganese (Mn) signal intensity was taken as the control value. The SOSA values were determined by measuring the reduction in RI values when 50 μ L samples were used in place of distilled water. Superoxide dismutase (SOD) enzyme was used as a standard. One milligram of pure SOD had a SOSA value equivalent to 3500 units. An SOSA measurement was not conducted on fruit and vegetable extracts, because ethanol used for the extraction may interfere with the measurement.

Survey for Beverage Consumption. A beverage consumption survey was conducted in the spring, summer, autumn, and winter seasons in 2004. Randomly selected male and female subjects (10–59 years old, n = 8768), except for single households, living within 30 km from the center of Tokyo, Osaka, Kyoto, and Kobe cities participated. Subjects recorded daily the amounts and kinds of all nonalcoholic beverages except for soup and tap water consumed over 7 days.

Statistical Analysis. All data were expressed as mean \pm standard deviation (SD). Statistical differences in correlation were examined with the Pearson's test.

RESULTS

Total Polyphenol Contents. The slope of the line from the standard curve of catechin, epicatechin, epicatechin gallate, epigallocatechin, epigallocatechin gallate, tannic acid, naringenin, gallic acid ethyl ester, and chlorogenic acid were 24.9, 28.7, 25.9, 20.0, 21.4, 21.9, 22.8, 20.1, and 15.5 (Abs 769 nm per mg/100 mL polyphenols), respectively. Just chlorogenic acid showed a less steep curve than the mean value of the other standard chemicals by 2 SD. We used chlorogenic acid as a

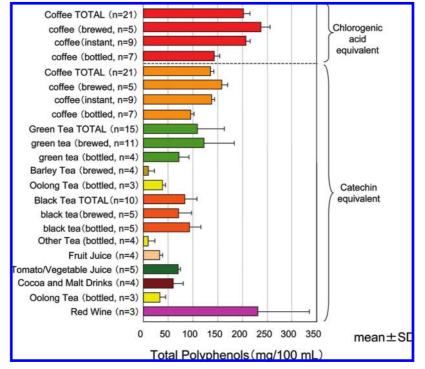


Figure 1. Total polyphenols in beverages.

Table 1. Total Polyphenol from Beverages Consumed in Japanese^a

| beverages | consumption of beverages | | averaged total polyphenol contents | daily total polyphenol consumption | |
|-----------------------------|--------------------------|--------|------------------------------------|------------------------------------|---------|
| | mL/day | (%) | mg/100 mL | mg/day | (%) |
| green tea | 253 ± 337 | (23%) | 115 | 292 ± 389 | (34%) |
| coffee | 213 ± 213 | (19%) | 200 | 426 ± 424 | (50%) |
| barley tea | 174 ± 325 | (16%) | 9 | 15 ± 28 | (2%) |
| oolong tea | 76 ± 214 | (7%) | 39 | 30 ± 84 | (4%) |
| fresh milk | 60 ± 127 | (5%) | | | · · · · |
| black tea | 59 ± 146 | (5%) | 96 | 57 ± 140 | (7%) |
| other tea | 53 ± 182 | (5%) | 8 | 4 ± 15 | (1%) |
| sports drinks | 52 ± 180 | (5%) | | | · · · · |
| carbonated drinks | 37 ± 127 | (3%) | | | |
| mineral water | 35 ± 136 | (3%) | | | |
| fruit juice | 32 ± 71 | (3%) | 34 | 11 ± 24 | (1%) |
| tomato/vegetable juice | 14 ± 56 | (1%) | 69 | 9 ± 38 | (1%) |
| cocoa/chocolate malt drinks | 10 ± 49 | (1%) | 62 | 6 ± 30 | (1%) |
| soy milk | 6 ± 30 | (0%) | 36 | 2 ± 11 | (0%) |
| others | 40 ± 147 | (4%) | | | () |
| total | 1113 ± 512 | (100%) | | 853 ± 512 | (100%) |

^a Weighted average using the consumption of sub categories of each beverage was calculated. 5-Caffeoyl quinic acid and catechin were used as standard for coffee and the other beverages, respectively.

standard for the detection of total polyphenol for coffee samples, and catechin for the other samples.

TP contents in beverages are shown in **Figure 1**. TP contents in 21 brewed, reconstituted instant, and commercially available bottled coffee samples excluding espresso coffee was approximately 200 mg/100 mL, which was similar to levels in red wines. Espresso coffee showed 1.5-5 times higher TP contents than the other types of coffee. Espresso coffee is usually taken at 30–60 mL per cup, and its per cup TP consumption was a similar level compared to brewed and instant, and bottled coffee is usually consumed at 140 and 190 mL, respectively. One serving of coffee similarly supplies approximately 300 mg of total polyphenol. Green and black tea showed approximately 100 mg/100 mL TP, vegetable juices and cocoa drinks showed 60-70 mg/100 mL TP, and the other beverages including Oolong tea and barley tea were less than those.

TP contents of fruit, vegetables, cacao mass, and black pepper were measured (**Table 1**). TP contents in the 20 major vegetables and 5 fruits, most commonly consumed in Japan, were 0 to 55 mg/100 g. Burdock showed the highest TP contents in vegetables, following by spinach, araceous, broccoli, and egg plant. Satsuma orange showed the highest TP contents in fruits. Black pepper and cacao mass, a main ingredient for chocolate, showed 733 and 7796 mg/100 g of TP contents.

Survey of Beverage Consumption. We conducted a survey of nonalcoholic beverage consumption in male and female Japanese living in two city areas, Tokyo and Osaka, from 10 to 59 years of age (n = 8768). The proportion of beverage consumption is shown in **Table 1** and **Figure 2A**. The average consumption of beverages was 1.11 ± 0.51 L/day. The most consumed beverage was green tea at 253 ± 337 mL/day which consisted of 23% of total consumption. Of the green tea, barley tea, and black tea, 78%, 96%, and 64% was prepared by brewing at home, respectively, and the remaining amounts were consumed ready to drink (RTD) from cans or PET bottles. Coffee had the second largest consumption at 213 ± 213 mL/day,

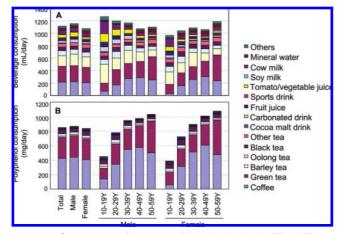


Figure 2. Consumption of beverages (A) and polyphenols (B) in different age and sex groups. Number of subjects of 10s, 20s, 30s, 40s, and 50s age groups were 783, 383, 1045, 950, and 735 in male group, and 894, 645, 1396, 1055, and 881 in female group, respectively.

sharing 19% of total beverage consumption. Brewed coffee, instant coffee, and bottled coffee shared 36%, 36%, and 12% in total coffee consumption, respectively. Decaffeinated instant coffee shared 0.5% of total instant coffee consumption. Proportion of consumption for the other beverages, barley tea, Oolong tea, black tea, fruit juice, and cocoa malt drinks was 16%, 7%, 5%, 3%, and 1%, respectively. In the fruit juice category, 100% concentrated juice consisted of a 58% share. Orange, apple, and grapefruits juices were the major varieties, consisting of 21%, 16%, 10% shares, respectively.

Consumption of beverages in the subgroups differing in age and sex is shown in **Figure 2A**. The proportion of beverage consumption was more influenced by age than by sex. Green tea was consumed largely in the group aged in their 50s, and coffee was consumed mainly by those in their 30s to 50s. Cow's milk and barley tea were more largely consumed in teenagers than adults.

Total Polyphenol Consumption. Total polyphenol consumption from beverages is shown in **Table 1**. Averaged TP contents were recalculated based on weighed consumption in subtype of beverages in the same category. On average, a Japanese person consumed 853 ± 512 mg/day of total polyphenols from beverages. Coffee was the largest source of total polyphenol consumption from beverages, consisting of 50% of daily TP consumption. Green tea and black tea consisted of 34% and 7%, respectively. Barley tea, Oolong tea, and cocoa were at most 4%.

TP consumption was influenced by age rather than by sex. TP consumption is higher in older age groups and reached more than 1 g/day in both male and female subjects aged more than 50 years old. TP consumption in the subjects aged less than 20 year old and 20-30 year old was a half and three-quarters smaller than adult subjects aged more than 30 years old, where consumption of coffee and green tea has a major impact on TP consumption.

Subjects were divided into four subgroups depending on the amount of coffee consumption (**Figure 3**). Green tea was similarly consumed by all four subgroups with different coffee consumption, and there was no compensation of TP intake from green tea in the low coffee consuming subgroups, resulting in TP consumption proportionally increasing with the increase in coffee consumption, and the subjects with high coffee consumption of more than 320 mL/day (fourth quartile) showed the largest TP consumption (**Figure 3B**).

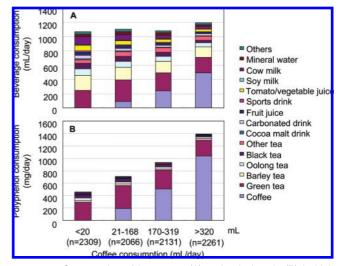


Figure 3. Consumption of beverages (A) and polyphenols (B) in high and low coffee consumers.

Antioxidant Activities. Three different types of antioxidant activities were measured for the beverage, fruit, and vegetable extracts samples (**Figure 4**). These activities, Cu reducing power (PAO), DPPH radical scavenging power (DPPH), and superoxide scavenging activity (SOSA), were positively correlated to the TP contents (p < 0.001). Coffee and teas were relatively high in all antioxidant activities compared to vegetable and fruit extracts.

DISCUSSION

Polyphenols are widely distributed in plants. Beverages except for dairy products and mineral water are mainly composed of extracts of plants; therefore, beverages are potentially a good source of polyphenols. In our survey, Japanese consumed on average 1.1 L of beverages everyday, of which the polyphenol contents were up to 853 mg/day. In some age groups such as 50-60 year olds, the average total polyphenol consumption was more than 1 g/day. Coffee contained the highest amount of polyphenols among all beverages at 200 mg/100 mL, and provided the largest polyphenol consumption at 426 mg/day. In fact, polyphenol is a large part of the solid in coffee extract. An instant coffee (NESCAFÉ, Nestlé Japan Ltd., Kobe) contains total polyphenols at 16% of the total solid, which is larger than components such as protein, minerals, and caffeine at 13%, 8%, and 3%, respectively (unpublished data). Green tea contains the second largest amount of polyphenol at 115 mg/100 mL, and it is the second largest source of polyphenol, giving 292 mg of polyphenol per day. These two beverages, coffee and green tea, are the two major sources of polyphenol in Japanese daily life and made up 50% and 34% of polyphenol consumption from beverages. Interestingly, we found that total polyphenol consumption of the upper quartile subjects with the highest coffee consumption was 3 times higher than that of the lowest quartile coffee consumption subgroup. The coffee heavy user group consumed green tea at a similar level in the other quartile subgroups with less coffee consumption. The large amount of polyphenols in coffee, as well as the characteristics of coffee that make it easy to consume in high amounts, may attribute to the supply of a large amount of antioxidants, which in turn may contribute to the prophylactic effect against some diseases. Contribution of polyphenol consumption from other beverages was relatively small, and less than 10% of the total polyphenol consumption in each category.

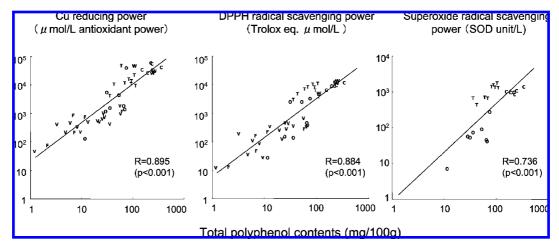


Figure 4. Correlation between total polyphenol contents and antioxidant activities (Cu reducing power, DPPH radical scavenging power, and superoxide scavenging power) in beverages and extracts from fruits and vegetables. C: coffeee; T: green tea, black tea and oolong tea; O: other beverages including juices and cocoa; W: red wine; V: vegetables; F: fruits.

 Table 2. Total Polyphenol from Major Vegetables, Fruits, and Other Foods

 Consumed in Japanese^a

| food | total polyphenol contents (mg/100 g) | | | | |
|------------------------|--------------------------------------|--|--|--|--|
| Vegetables | | | | | |
| burdock | 49.3 ± 9.3 | | | | |
| spinach | 41.6 ± 13.7 | | | | |
| araceous | 35.5 ± 26.1 | | | | |
| broccoli | 35.2 ± 8.5 | | | | |
| egg plant | 30.7 ± 2.8 | | | | |
| onion | 16.3 ± 10.2 | | | | |
| soybean (boiled) | 15.1 ± 21.4 | | | | |
| Welsh onion | 15.0 ± 12.6 | | | | |
| corn | 13.2 ± 11.5 | | | | |
| radish | 13.2 ± 10.6 | | | | |
| bamboo shoot (boiled) | 11.7 ± 9.3 | | | | |
| green pepper | $11.7 \pm 10.9 \\ 5.8 \pm 6.7$ | | | | |
| tomato | 5.8 ± 0.7 5.4 ± 5.1 | | | | |
| pumpkin cabbage | 5.4 ± 5.1 5.1 ± 3.5 | | | | |
| Chinese cabbage | 3.1 ± 3.3 4.5 ± 3.1 | | | | |
| potato | <0.1 | | | | |
| cucumber | <0.1 | | | | |
| carrot | <0.1 | | | | |
| lettuce | <0.1 | | | | |
| | | | | | |
| total vegetables | 19.3 ± 14.3 | | | | |
| Fruits | | | | | |
| Satsuma orange | 54.9 ± 6.3 | | | | |
| banana | 19.2 ± 6.8 | | | | |
| apple | 4.8 ± 1.8 | | | | |
| grape (red) | 11.7 ± 13.3 | | | | |
| melon | 2.4 ± 2.7 | | | | |
| total fruits | 18.6 ± 21.3 | | | | |
| | Others | | | | |
| black pepper | 733 + 493 | | | | |
| chocolate (cacao mass) | 733 ± 493 7796 ± 1680 | | | | |
| red wine | 230 ± 78 | | | | |
| | 200 - 10 | | | | |

^a Catechin was used as a standard.

Some foods such as vegetables, fruits, chocolate, herbs, spices, alcohol (e.g., wine and beer) and cereals as well as beverages (23-31) contain polyphenols. Based on statistical data issued by the Japanese government in 2004 on national production, the import/export of fruit and vegetables and population, the per capita consumption of the 20 top major vegetables and 5 fruits was 256 g/day (11 ± 12 , 1-50 g/day) and 56 g/day (11 ± 5 , 3-16 g/day), respectively. In the Tokyo Metropolitan market, the top 20 vegetables and 5 fruits made up 78% and 54% of total trade

tonnage, respectively (32), suggesting that a large part of their consumption is included in the selected vegetables and fruits in the Japanese diet. Based on our present results and this statistical data, the largest fruit and vegetable TP consumption was Satsuma oranges and onions at 9 and 4 mg/day, respectively, and the total top 20 vegetables and top 5 fruits give 19 mg and 14 mg of TP consumption, which was 10 times lower than that for beverages. Per capita polyphenol consumption of cacao mass and black pepper were estimated at 0.8 and 0.2 g/day based on the import volume (33, 34). Consumption of red wine was estimated at 3.0 mL/day considering red wine to be 60% of the total annual consumption of wine (35). Based on these statistics, consumption of polyphenol from black pepper, chocolate (cacao mass), and red wine was calculated at 2, 67, and 7 mg/day. Chocolate is the largest source of polyphenol among nonbeverage categories, and its amount was still 10 times smaller than that of total beverages, and 5 times smaller than that of coffee. It is difficult to estimate the total consumption of polyphenols from nonbeverages, because of their huge variety and the lack of information on their consumption. In this study, we did not include some nonbeverage foods reported rich in polyphenols or high in antioxidant activity such as herbs, dry fruits, berries, and beans (36-39). However, the consumption of these foods in Japan is not so large. For instance, Japanese frequently consume soy products, but the consumption of isoflavones among Japanese is reported at 18 mg/day (40). Our results suggest that beverages are the largest source of polyphenols in the Japanese diet.

In this study, we applied a modified Folin-Ciocalteu method for the measurement of total polyphenols. The advantage of this method is that it removes interference of the reduced sugars including vitamin C using a reverse-phase column chromatography. According to our results, approximately half of the colorimetric values in the Folin-Ciocalteu method came from interference, which corresponds to the results of George et al. on fruit juices (24). Polyphenol contents from fruit and vegetables shown in this study were small compared to the results of the U.S. study (22). Methodological difference may partially cause differences in the analytical values, as well as the difference between spices, source, and seasonality (23). Coffee contains a mixture of coffee polyphenols including 5-caffeoyl quinic acid (CQA), and other chlorogenic acid groups such as 3- and 4-CQAs, 3-, 4-, and 5-fluoyl quinic acids, and 3,4- and 3,5-diCQAs, and largely polymerized polyphenols called melanoidins, all of which have antioxidant activity (29, 41). 5-CQA and the other simple 7 chlorogenic acids made up 10% and 20% of total polyphenols in the instant coffee sample (unpublished data). Total polyphenol contents showed good correlation against three kinds of antioxidant activities, Cu+ reducing, DPPH radical scavenging, and superoxide scavenging activities, suggesting that the total polyphenol values measured in this study are meaningful and reflect the total in vitro antioxidant properties. Bioavailability of polyphenols is different for each polyphenol molecule and may influence bioefficacy in humans. Catechins from tea, chlorogenic acids from coffee, and the deliveries metabolized by intestinal flora are shown to be absorbed in humans (26, 42). High consumption of coffee and its bioavailability, and tea polyphenols may contribute to provide functionality for risk reduction of some diseases among humans.

In conclusion, this study was conducted to reveal the total polyphenol consumption from all nonalcohol beverages and some major foods containing polyphenols in the Japanese diet. Polyphenol consumption from beverages was large and reached more than one gram per day in middle-aged adults, half of this amount coming from coffee. Beverages, especially coffee and tea, are a large source of polyphenols in the daily life of the Japanese population.

ABBREVIATIONS USED

DPPH: 1,1-diphenyl-2-picrylhydrazyl; ESR: electron-spin resonance; HPLC: high performance liquid column chromatography; PAO: test kit for potential antioxidant SOD: superoxide dismutase; SOSA: superoxide scavenging activity; TP: total polyphenol.

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LITERATURE CITED

- Rice-Evans, C. A.; Miller, N. J.; Paganga, G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *<u>Free Radic.</u> Biol. Med.* **1996**, *20*, 933–956.
- (2) Arts, I. C.; Hollman, P. C. Polyphenols and disease risk in epidemiologic studies. <u>Am. J. Clin. Nutr</u>. 2005, 81, 317S–325S, 1 Suppl.
- (3) van Dam, R. H.; Hu, F. B. Coffee consumption and risk of Type 2 diabetes: A systematic review. JAMA 2005, 294, 97–104.
- (4) Yamaji, T.; Mizoue, T.; Tabata, S.; Ogawa, S.; Yamaguchi, K.; Shimizu, E.; Mineshita, M.; Kono, S. Coffee consumption and glucose tolerance status in middle-aged Japanese men. <u>*Diabeto-logia*</u> 2004, 47, 2145–2151.
- (5) Iso, H.; Date, C.; Wakai, K.; Fukui, M.; Tamakoshi, A. JACC Study Group. The relationship between green tea and total caffeine intake and risk for self-reported type 2 diabetes among Japanese adults. *Ann. Intern. Med.* **2006**, *144*, 554–562.
- (6) Tverdal, A.; Skurtveit, S. Coffee intake and mortality from liver cirrhosis. <u>Ann. Epidemiol</u>. 2003, 13, 419–423.
- (7) Ruhl, C. E.; Everhart, J. E. Coffee and tea consumption are associated with a lower incidence of chronic liver disease in the United States. *Gastroenterol.* 2005, *129*, 1928–1936.
- (8) Shimazu, T.; Tsubono, Y.; Kuriyama, S.; Ohmori, K.; Koizumi, Y.; Nishino, Y.; Shibuya, D.; Tsuji, I. Coffee consumption and the risk of primary liver cancer: pooled analysis of two prospective studies in Japan. *Int. J. Cancers* **2005**, *116*, 150–154.
- (9) Inoue, M.; Yoshimi, I.; Sobue, T.; Tsugane, S. JPHC Study Group. Influence of coffee drinking on subsequent risk of hepatocellular

carcinoma: a prospective study in Japan. <u>J. Natl. Cancer Inst</u>. 2005, 97, 293–300.

- (10) Giovannucci, E. Meta-analysis of coffee consumption and risk of colorectal cancer. <u>Am. J. Epidemiol</u>. **1998**, 147, 1043–1052.
- (11) Andersen, L. F.; Jacobs, D. R., Jr.; Carlsen, M. H.; Blomhoff, R. Consumption of coffee is associated with reduced risk of death attributed to inflammatory and cardiovascular diseases in the Iowa Women's Health Study. <u>Am. J. Clin. Nutr.</u> 2006, 83, 1039–1046.
- (12) Baba, S.; Osakabe, N.; Kato, Y.; Natsume, M.; Yasuda, A.; Kido, T.; Fukuda, K.; Muto, Y.; Kondo, K. Continuous intake of polyphenolic compounds containing cocoa powder reduces LDL oxidative susceptibility and has beneficial effects on plasma HDLcholesterol concentrations in humans. <u>Am. J. Clin. Nutr</u>. 2007, 85, 709–717.
- (13) Buijsse, B.; Feskens, E. J. M.; Kok, F. J.; Kromhout, D. Cocoa intake, blood pressure, and cardiovascular mortality. <u>Arch. Int.</u> <u>Med.</u> 2006, 166, 411–417.
- (14) Schroeter, H.; Heiss, C.; Balzer, J.; Kleinbongard, P.; Keen, C. L.; Hollenberg, N. K.; Sies, H.; Kwik-Uribe, C.; Schmitz, H. H.; Kelm, M. (–)Epicatechin mediates beneficial effects of flavanolrich cocoa on vascular function in humans. *Proc. Natl. Acad. Sci.* <u>U. S. A</u>. 2006, 108, 1024–1029.
- (15) Kuriyama, S.; Shimazu, T.; Ohmori, K.; Kikuchi, N.; Nakaya, N.; Nishino, Y.; Tsubono, Y.; Tsuji, I. Green tea consumption and mortality due to cardiovascular disease, cancer, and all causes in Japan: The Ohsaki Study. *JAMA* 2006, 296, 1255–1265.
- (16) Ames, B. N.; Shigenaga, M. K.; Hagen, T. M. Oxidants, antioxidants, and the degenerative diseases of aging. <u>Proc. Natl.</u> <u>Acad. Sci. U. S. A.</u> 1993, 90, 7915–7922.
- (17) Block, G. Epidemiologic evidence regarding vitamin C and cancer. *Am. J. Clin. Nutr.* **1991**, *54*, 1310S–1314S, 6 Suppl.
- (18) Gillman, M. W.; Cupples, L. A.; Gagnon, D.; Posner, B. M.; Ellison, R. C.; Castelli, W. P.; Wolf, P. A. Protective effect of fruits and vegetables on development of stroke in men. <u>JAMA</u> **1995**, 273, 1113–1117.
- (19) Scalbert, A.; Williamson, G. Dietary intake and bioavailability of polyphenols. J. Nutr. 2000, 130, 2073S–2085S.
- (20) Svilaas, A.; Sakhi, A. K.; Andersen, L. F.; Svilaas, T.; Strom, E. C., Jr.; Ose, L.; Blomhoff, R. Intakes of antioxidants in coffee, wine, and vegetables are correlated with plasma carotenoids in humans. J. Nutr. 2004, 134, 562–567.
- (21) Vinson, J. A.; Hao, Y.; Su, X.; Zubik, L. Phenol antioxidant quantity and quality in foods; vegetables. <u>J. Agric. Food Chem</u>. 1998, 46, 3630–3634.
- (22) Vinson, J. A.; Su, X.; Zubik, L.; Bose, P. Phenol antioxidant quantity and quality in foods: fruits. <u>J. Agric. Food. Chem</u>. 2001, 49, 5315–5321.
- (23) Hertog, M. G. L.; Hollman, P. C. H.; Katan, M. B. Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. <u>J. Agric. Food</u> <u>Chem.</u> 1992, 40, 2379–2383.
- (24) George, S.; Brat, P.; Alter, P.; Amiot, M. J. Rapid determination of polyphenols and vitamin C in plant-derived products. <u>J. Agric.</u> Food Chem. 2005, 53, 1370–1373.
- (25) Sakakibara, H.; Honda, Y.; Nakagawa, S.; Ashida, H.; Kanazawa, K. Simultaneous determination of all polyphenols in vegetables, fruits, and teas. *J. Agric. Food Chem.* **2003**, *51*, 571–581.
- (26) Manach, C.; Scalbert, A.; Morand, C.; Remesy, C.; Jimenez, L. Polyphenols: food sources and bioavailability. <u>Am. J. Clin. Nutr.</u> 2004, 79, 727–747.
- (27) Justesen, U.; Knuthsen, P.; Leth, T. Quantitative analysis of flavonols, flavones, and flavanones in fruits, vegetables and beverages by high-performance liquid chromatography with photodiode array and mass spectrometric detection. <u>J. Chromatogr. A</u>. 1998, 799, 101–110.
- (28) Arts, I. C.; van de Putte, B.; Hollman, P. C. Catechin contents of foods commonly consumed in The Netherlands. 1. Fruits, vegetables, staple foods, and processed foods. <u>J. Agric. Food</u> <u>Chem.</u> 2000, 48, 1746–1751.

- (29) Clifford, M. N. Chlorogenic acids and other cinnamates 227 nature, occurrence and dietary burden. <u>J. Sci. Food Agric</u>. **1999**, 79, 362– 372.
- (30) Clifford, M. N. Chlorogenic acids and other cinnamates-nature, occurrence, dietary burden, absorption and metabolism. <u>J. Sci.</u> <u>Food Agric</u>. 2000, 80, 1033–1043.
- (31) Sato, M.; Ramarathnam, N.; Suzuki, Y.; Ohkubo, T.; Takeuchi, M.; Ochi, H. Varietal differences in the phenolic content and superoxide radical scavenging potential of wines from different sources. *J. Agric. Food Chem.* **1986**, *44*, 37–41.
- (32) Tokyo Vegetable and Fruit Information Center, Tokyo Metropolitan Market Vegetable and Fruit Annual Report 2004.
- (33) Takase, K. Pure chocolate leads the chocolate market. *Alcohol Beverages Statistics Monthly* 2005, *12*, 38–44 (in Japanese).
- (34) Ministry of Finance, Trade Statics in Japan, 2004.
- (35) National Tax Administration Agency, Yearbook of Statistics, 2004.
- (36) Katsube, T.; Tabata, H.; Ohta, Y.; Yamasaki, Y.; Anuurad, E.; Shiwaku, K.; Yamane, Y. Screening for antioxidant activity in edible plant products: comparison of low-density lipoprotein oxidation assay, DPPH radical scavenging assay, and Folin-Ciocalteu assay. *J. Agric. Food Chem.* 2004, *52*, 2391–2396.
- (37) Pellegrini, N.; Serafini, M.; Colombi, B.; Del Rio, D.; Salvatore, S.; Bianchi, M.; Brighenti, F. Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assays. *J. Nutr.* **2003**, *133*, 2812–2819.

- (38) Vinson, J. A.; Zubik, L.; Bose, P.; Samman, N.; Proch, J. Dried fruits: excellent in vitro and in vivo antioxidants. <u>J. Am. Coll.</u> <u>Nutr.</u> 2005, 24, 44–50.
- (39) Franke, A. A.; Custer, L. J.; Cerna, C. M.; Narala, K. K. Quantification of phytoestrogens in legumes by HPLC. <u>J. Agric.</u> *Food Chem.* **1994**, *42*, 1905–1913.
- (40) Toda, T.; Tamura, J.; Okudaira, T. Contents of isoflavones in commercially-supplied soy products. *FFI J.* **1997**, *172*, 83–89.
- (41) del Castillo, M. D.; Ames, J. M.; Gordon, M. H. Effect of roasting on the antioxidant activity of coffee brews. *J. Agric. Food Chem.* 2002, *50*, 3698–3703.
- (42) Olthof, M. R.; Hollman, P. C.; Buijsman, M. N.; van Amelsvoort, J. M.; Katan, M. B. Chlorogenic acid, quercetin-3-rutinoside and black tea phenols are extensively metabolized in humans. <u>J. Nutr.</u> 2003, 133, 1806–1814.

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