Assessing self-reported green tea and coffee consumption by food frequency questionnaire and food record and their association with polyphenol biomarkers in Japanese women

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Running title: Assessment of self-reported nutrition intake

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Data collection and analyses were done by R.T., H.A., A.H., N.H., A.I., L.T., and A.H.L. The manuscript was prepared by R.T., H.A., A.H., A.T., and A.H.L. The project was led and managed by R.T. and A.H.L

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ABSTRACT

**Background and Objectives:** Despite the demonstrated protective effects of green tea and coffee intake against several chronic diseases, finding between studies have not been consistent. One potential reason of this discrepancy is the imprecision in the measurement of tea or coffee consumption using food frequency questionnaire (FFQ) and food record (FR) in epidemiological studies. **Methods and Study Design:** In a sample of 57 healthy Japanese women, intake of green tea and coffee was estimated by a validated FFQ and a 3-day FR, while their plasma and urine concentrations of polyphenol biomarkers were measured by HPLC. The polyphenols assessed included (−)-epigallocatechin gallate (EGCG), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC) and (−)-epicatechin (EC), caffeic acid (CA) and chlorogenic acid (CGA). **Results:** Green tea consumption estimated by FFQ and FR showed moderate association, while strong association was detected for coffee consumption. Urinary green tea polyphenol concentrations were moderately-strongly associated with FR-estimated intake, while the associations were weak with FFQ. Similarly, coffee polyphenols in urine were moderately associated with FR-estimated coffee intake, whereas FFQ showed poor correlation. The associations between urinary and plasma polyphenols ranged from moderate to high. **Conclusions:** The results indicated that firstly, the FFQ tends to overestimate green tea intake. Secondly, the urinary polyphenols are preferred over plasma polyphenols as a potential surrogate marker of the short-term green tea and coffee intake, while their use as an indicator of long-term consumption is not reliable.

**Key Words:** polyphenols, green tea, coffee, food frequency questionnaire, food record

INTRODUCTION

Green tea and coffee have garnered much research attention in recent years due to their possible role in lowering the risk of cardiovascular disease, type-2 diabetes and some cancers, which has been attributed to the antioxidant, anti-inflammatory, anti-atherogenic, and anti-carcinogenic bioactivity of polyphenols contained in these two beverages. **However,** the results have not been consistent between studies regarding the beneficial effects of green tea and coffee. One possible reason is the inherent imprecision in the measurement of green tea and coffee intake by using self-reported food frequency questionnaires (FFQ) and food records (FR).

Self-reported FFQ and FR are instruments commonly used in nutritional epidemiology to measure the dietary intake of individuals. Each instrument has limitations and is designed for
measuring intake under differing conditions.\textsuperscript{15,16} FFQs record frequency and quantity of habitual dietary intake from memory over an extended period within the recent past, such as the previous year. Alternatively, FRs diarize their intake over a specific brief period of several days as it occurs. The FFQ is known to be particularly prone to measurement errors,\textsuperscript{17} presumably due to the sheer cognitive difficulty of accurately recalling past intakes over an extended period. Conversely, there is no guarantee that short-term intake recorded via a FR can represent an individual’s habitual dietary intake, while it captures the cross-sectional food and beverage consumption in better accuracy. Additionally, recalling customary intake over time, as in the case of FFQ, does not necessarily reflect the actual long-term consumption pattern, since the dietary habits during the previous year can change substantially from the past. Many questions remain regarding the accuracy and reliability of FFQs and FRs.\textsuperscript{18,19} Since the accurate measurement of dietary intake is vital in establishing the health benefits of dietary exposures,\textsuperscript{20} it is necessary to assess the application of FFQ and FR instruments, especially in the context of green tea and coffee consumption.\textsuperscript{21}

Both green tea and coffee contain a complex array of compounds that exhibit beneficial effects on metabolic diseases. Green tea is a rich source of flavanols, primarily (\textendash)epigallocatechin gallate (EGCG), (\textendash)epicatechin gallate (ECG), (\textendash)epigallocatechin (EGC) and (\textendash)epicatechin (EC).\textsuperscript{21\textendash}23 Coffee provides a major source of chlorogenic acids (CGA), a family of esters formed through esterification of trans-cinnamic acids (caffeic, ferulic and p-coumaric acid) with quinic acid.\textsuperscript{24,25} Measuring intake of tea and coffee constituent compounds is a challenge, due to the diversity in methods of preparation, chemical composition, quantity or concentration/intensity,\textsuperscript{26} as well as uncertainties regarding compound degradation.\textsuperscript{27}

The present study aims to ascertain the correlation between FFQ and FR in measuring green tea and coffee consumption, and the extent of their association with the polyphenol biomarkers in plasma and urine.

**MATERIALS AND METHODS**

**Subjects**

A total of 57 Japanese women participated in this cross-sectional study conducted between April and August 2014. The Japanese adult population was considered suitable because habitual green tea consumption is a characteristic of the Japanese diet, while coffee consumption is also increasingly common.\textsuperscript{28} Recruitment of participants took place in Himeji.
City within Hyogo Prefecture through the assistance of Tsunashimakai Kosei Hospital (32 subjects) and University of Hyogo (25 subjects).

The study was restricted to females 35 years of age and above in order to limit potential variations due to gender, as well as to select a representative sample of adult women by which patterns of long-term tea or coffee consumption behaviour have been established. The fulfilment of inclusion criteria was achieved through a screening interview. Exclusion applied if individuals were currently on prescription for a chronic condition or had substantially modified their diet within the past year.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the Curtin Human Research Ethics Committee and University of Hyogo Research Ethic Committee. Written informed consent was obtained from all subjects.

Assessment of green tea and coffee consumption
Information concerning dietary intake over the past twelve months was collected using a self-administered FFQ, previously validated by the Japan Public Health Centre-based prospective study. Subjects were asked to indicate the frequency, duration, number of cups, and cup size of coffee and green tea intake in order to estimate the total consumption of green tea/coffee in the last 12 months. Missing or inconsistent responses were subsequently rectified through a face-to-face interview by one of our investigators.

During the same period, participants were requested to commence a 3-day FR four days prior to the meeting. Specific instructions on recording green tea and coffee intake were provided in detail. Their completed FRs were checked carefully for erroneous entries during the interview and unknown items were clarified. Quantity of teas and coffees consumed during the three days were also verified with photos provided by the participants.

Measurement of urine and plasma polyphenols
First morning void urine was collected and venous blood sampling was performed on the day of interview. High performance liquid chromatography (HPLC; Agilent 1100 LC with binary pump) coupled with tandem mass spectrometry (MS/MS) (Applied Biosystems Sciex API 3000) were utilized to determine polyphenol concentrations in both urine and plasma as detailed previously. Standards of EGCG, EGC, ECG, EC, caffeic acid (CA), CGA and ethyl gallate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Peaks for each compound were identified by comparing retention times and mass spectral data with
respective standards and published data,\textsuperscript{25,30-32} utilizing Analyst v1.6 software (ABSciex) for management.

**Statistical analysis**

Most of the variables were non-normally distributed as indicated by the D’Agostino-Pearson normality test and other diagnostics. Therefore, median and inter-quartile range were used to summarize their distributions. The nonparametric Spearman’s rank correlation was calculated to ascertain the relationship between polyphenol metabolites of tea or coffee recovered in plasma and urine samples, and intake amounts obtained by FFQ or FR. All statistical analyses were performed using the Stata software Release 13 (StataCorp. 2013. College Station, TX).

**RESULTS**

Table 1 presents the characteristics of our sample of 57 generally healthy women, whose mean age was 52 (SD=8.0) years. According to the self-administered FFQ, the majority of participants had been drinking green tea for more than 20 years (61.4%), whilst others reported between 10-20 years (8.8%) and 2-3 years (3.5%). An average quantity of dried tea leaves used to infuse green tea was one tea spoon (approximately 2 g) per cup (180 mL). Over one-third (38.6%) of participants drank green tea every day, otherwise green tea was consumed on at least 3 days per week (61.4%). About a quarter (26.3%) of women rarely or never consumed green tea. Number of cups per day (standardized at 150 mL per cup) ranged from less than 1 to greater than 7 cups, with mean consumption of 2.3±2.4 cups. However, the FR found a lower mean consumption of 1.0±1.7 cups, with more than half of participants reporting no green tea consumption during the 3-day period. Those that did drink green tea generally reported less than 3 cups. The Spearman’s rank correlation between FFQ and FR for green tea consumption frequency was moderate (r=0.414, \(p=0.001\)).

In FFQ, most participants reported drinking coffee for more than 20 years (84.2%) on a daily basis (68.4%) or at least 3 days a week (84.2%). Both FFQs and FRs reported 7% of women were non-coffee drinkers. Similar to green tea consumption, average number of cups (standardized at 200 mL per cup) consumed per day was higher by FFQ (2.9±2.0 cups) than FR (1.5±1.0 cups). FR indicated that two-third (68%) of participants drank 2 cups or less coffee but 63% stated more than 2 cups per day habitually as recorded by the FFQ, suggesting possible overestimation by the latter. Nevertheless, coffee consumption frequency as reported by these two methods was strongly correlated (r=0.566, \(p<0.001\)).
Figure 1 plots the Spearman’s rank correlations between green tea consumption, as estimated by FFQ and FR, and major green tea catechins in both plasma and urine (also see Supplementary Table 1). Overall, stronger associations were observed for FR than FFQ. The correlation coefficients between catechins and FFQ appeared to be low to moderate, with urinary EGCG exhibiting the strongest positive association. Similarly, the highest correlation was observed between urinary EGCG and green tea consumption estimated from FR. It is evident that urinary catechins showed stronger associations with FR-green tea consumption than plasma catechins. It is also noted that EC detected in the urine was least correlated with FR when compared to the other three catechins.

Figure 2 and Supplementary Table 2 present the Spearman’s rank correlations between coffee consumption and coffee polyphenols, CA and CGA, which were generally lower than the corresponding coefficients for green tea. Overall, urinary CA and CGA were moderately correlated with FR coffee consumption, whereas the associations with coffee polyphenols were weaker for FFQ when compared to FR.

Finally, Figure 3 summarises the correlations between urinary and plasma polyphenols (also see Supplementary Table 3). It can be seen that the associations ranged from moderate (CA, EC, ECG) to high (EGCG, EGC), with the exception of CGA.

**DISCUSSION**

Green tea and coffee are beverages that are consumed across the globe, and are suggested to have various health benefits due to their high polyphenol content.\(^1\),\(^9\),\(^33\) However, despite decades of research, results are still inconclusive and the inconsistencies may be attributed to the methodological limitations in measuring habitual intake.

In this study, we investigated the associations between green tea and coffee intakes as measured by the self-reported FR and FFQ, and plasma and urinary concentrations of green tea and coffee polyphenols in Japanese women. Japan is among the few countries with a high consumption of both green tea and coffee, which is an ideal and appropriate setting for the purpose of this study. Indeed, our results from FFQ indicated that only 26% and 7% of participants did not or rarely consumed green tea and coffee, respectively. Consistently, FR results revealed that 7% of the participants did not drink coffee during the 3-day FR period. The consistency between FFQ and FR in estimating coffee intake was also evident from the high correlation coefficient. In comparison, the correlation between FFQ and FR was only moderate for green tea consumption, since the FR reported no green tea intake by more than 50% of the participants. This may be caused by a misunderstanding towards the classification
of ‘green tea’. Green tea is typically prepared by infusing hot water with dried leaves of Camellia sinesis. The green tea leaves are dried by steaming or pan-frying, which preserves the catechins at highest content, whereas black tea and oolong tea are fermented and consequently contain lesser amount of catechins. In addition, other tea types such as herbal teas and barley teas are commonly available in Japan, which contain no catechins at all, but can be mistakenly included in green tea consumption by the participants when completing their FFQ, whereas such misclassification is unlikely to occur in FR where the types of beverages are recorded in detail. Consistently, the observed correlations between plasma/urinary catechins and green tea consumption were substantially weaker by FFQ than their FR counterparts. These results collectively suggest that green tea intake may be over-reported by FFQ as a consequence of potential misclassification by the Japanese women.

The correlation analysis also revealed the stronger association between green tea consumption estimated by FR and urinary green tea catechins than plasma catechins, indicating that the excretion rate of catechins may reflect the green tea intake more accurately. Within the four catechins measured, EGCG exhibited the highest correlation, suggesting the possible use of first void urinary EGCG concentration as a surrogate biomarker for short-term green tea consumption. Similarly, the urinary concentrations of coffee polyphenols showed stronger association with the coffee intake via FR when compared to the plasma polyphenols, albeit at moderate level only and thus their use as markers of short-term coffee intake may be limited. These findings are consistent with previous studies, in which 24-hour urine samples were analyzed, and high correlations were found between urinary EC and CGA and the consumptions of green tea and coffee, respectively. Additionally, Mennen et al examined the association between polyphenol-rich food intake, measured using a two-day FR, and spot urine samples, and found that coffee intake was positively correlated with CGA and CA.

The observed correlations between urinary and plasma polyphenols varied from weak to high. In addition, the plasma concentrations of these polyphenols were less correlated with the 3-day consumption than their corresponding urinary concentrations, indicating significant inter-individual variations in the bioavailability, metabolism and retention of green tea and coffee polyphenols within the body. Indeed, studies have reported that dietary intake does not truly reflect systemic exposure, as extensive metabolism and inter-individual differences in bioavailability can significantly change the polyphenolic profile that naturally occurs in green tea leaves and coffee beans. There is still limited knowledge regarding the extent of absorption and metabolic rates of individual polyphenols from particular foods, hence the
need to evaluate current methods of dietary intake assessment for green tea and coffee polyphenols, using objective tools such as biomarkers.\textsuperscript{40,41} It is important to measure polyphenol concentrations in urine and/or plasma in order to determine systemic exposure, which has more biological and epidemiological significance than consumption alone.\textsuperscript{42}

Whilst this study has provided useful information, there are several limitations. The present study recruited a convenience sample of generally healthy Japanese women so that findings cannot be extrapolated to all adults as well as other populations. Also, in view of the available sample size, only univariate correlation analyses were performed to assess the apparent associations without accounting for the effect of potential demographic, lifestyle and anthropometric confounders. Replications with larger representative samples of both men and women are recommended in future investigations.

In conclusion, a number of issues should be considered when conducting epidemiological studies of green tea and coffee. Firstly, green tea and coffee consumption, as estimated by FR and FFQ, agree reasonably well, although the latter tend to overestimate green tea intake by including other teas as green tea. Secondly, urinary polyphenols are preferred over plasma polyphenols as a potential surrogate marker of the short-term green tea and coffee intake, especially for green tea; however, their use as an indicator of long-term consumption is not reliable. Lastly, because of the substantial inter-individual differences in bioavailability and metabolisms, the plasma concentrations of polyphenols should be measured together with the self-reported FFQ and FR in order to more accurately ascertain the physiological effects of green tea and coffee polyphenols.

**ACKNOWLEDGEMENTS**

Naoko Uemura and Yu Fujiwara deserve thanks for their assistance with data collection and polyphenol analysis. The authors also appreciate the assistance of Kenji Matsumoto, Director of Tsunashimakai Kosei Hospital, in facilitating the study.

**CONFLICT OF INTEREST AND FUNDING DISCLOSURE**

The authors declare no personal or financial conflicts of interest. Financial support was provided by the School of Biomedical Sciences, Curtin University, in addition to the first author’s Research Fellowship provided by the National Health and Medical Research Council of Australia.
REFERENCES

Table 1. Characteristics of participants (n = 57)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean±SD</th>
</tr>
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<tbody>
<tr>
<td>Age (years)</td>
<td>52.0±8.0</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>54.2±9.0</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.5±4.1</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>72.0±9.5</td>
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<tr>
<td>Hip circumference (cm)</td>
<td>89.5±7.1</td>
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<tr>
<td>Menopausal (%)</td>
<td>27 (47.4%)</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dL)</td>
<td>130.25±30.65</td>
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<tr>
<td>HDL-cholesterol (mg/dL)</td>
<td>71.21±17.19</td>
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<tr>
<td>Triglyceride (mg/dL)</td>
<td>101.82±94.09</td>
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<tr>
<td>Fasting blood glucose (mg/dL)</td>
<td>94.18±36.55</td>
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<tr>
<td>Insulin (μU/mL)</td>
<td>4.82±3.13</td>
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<tr>
<td>HbA1c (%)</td>
<td>5.44±0.96</td>
</tr>
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</table>

BMI: body mass index; LDL: low density lipoprotein; HDL: high density lipoprotein; HbA1c: glycated haemoglobin.
Figure 1. Spearman’s correlation coefficients between green tea consumption, as measured by food frequency questionnaire (FFQ) and 3-day food record (FR), and plasma/urinary polyphenol concentrations of (-)-epigallocatechin gallate (EGCG), (-)-epicatechin gallate (ECG), (-)-epigallocatechin (EGC) and (-)-epicatechin (EC).

Figure 2. Spearman’s correlation coefficients between coffee consumption, as measured by food frequency questionnaire (FFQ) and 3-day food record (FR), and plasma/urinary caffeic acid (CA) and chlorogenic acid (CGA) concentrations.

Figure 3. Spearman’s correlation coefficients between plasma and urinary concentrations of coffee and green tea polyphenols.
### Supplementary Table 1. Spearman’s correlation between green tea consumption and plasma/urine polyphenol concentrations

<table>
<thead>
<tr>
<th>Polyphenol</th>
<th>FFQ Plasma</th>
<th>UFQ Urine</th>
<th>FR Plasma</th>
<th>FR Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGCG</td>
<td>0.24 (p=0.08)</td>
<td>0.30 (p=0.02)</td>
<td>0.50 (p&lt;0.001)</td>
<td>0.53 (p&lt;0.001)</td>
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<tr>
<td>EGC</td>
<td>0.24 (p=0.07)</td>
<td>0.24 (p=0.07)</td>
<td>0.46 (p&lt;0.001)</td>
<td>0.50 (p&lt;0.001)</td>
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<tr>
<td>ECG</td>
<td>0.17 (p=0.20)</td>
<td>0.27 (p=0.04)</td>
<td>0.41 (p&lt;0.001)</td>
<td>0.50 (p&lt;0.001)</td>
</tr>
<tr>
<td>EC</td>
<td>0.23 (p=0.09)</td>
<td>0.19 (p=0.16)</td>
<td>0.22 (p=0.11)</td>
<td>0.44 (p&lt;0.001)</td>
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</table>

### Supplementary Table 2. Spearman’s correlation between coffee consumption and plasma/urine polyphenol concentrations

<table>
<thead>
<tr>
<th>Polyphenol</th>
<th>FFQ Plasma</th>
<th>UFQ Urine</th>
<th>FR Plasma</th>
<th>FR Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA</td>
<td>0.11 (p=0.42)</td>
<td>0.15 (p=0.27)</td>
<td>0.22 (p=0.09)</td>
<td>0.38 (p&lt;0.001)</td>
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<tr>
<td>CGA</td>
<td>0.10 (p=0.47)</td>
<td>0.09 (p=0.51)</td>
<td>0.04 (p=0.80)</td>
<td>0.38 (p&lt;0.001)</td>
</tr>
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</table>

### Supplementary Table 3. Spearman’s correlation between plasma and urinary polyphenol concentrations

<table>
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<tr>
<th>Polyphenol</th>
<th>Spearman’s correlation coefficient</th>
<th>p value</th>
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<tbody>
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<td>0.63</td>
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<td>EC</td>
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<td>0.0029</td>
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<tr>
<td>CA</td>
<td>0.33</td>
<td>0.0134</td>
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<tr>
<td>CGA</td>
<td>-0.02</td>
<td>0.904</td>
</tr>
</tbody>
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