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Consumption of Green Tea Causes Rapid Increase in Plasma Antioxidant Power in Humans

I. F. F. Benzie, Y. T. Szeto, J. J. Strain, and B. Tomlinson

Abstract: *Green tea contains polyphenolic antioxidants that have shown anticarcinogenic properties in animal and* in vitro *experimental studies. Current data regarding absorption and bioavailability of tea antioxidants in humans, however, are conflicting. In this study, plasma and urine antioxidant power after ingestion of green tea was measured using the ferric reducing/antioxidant power (FRAP) assay (US patent pending) to assess absorption, systemic distribution, and renal excretion of green tea antioxidants in healthy adults. Results showed that absorption of green tea antioxidants was rapid, with peak increase in plasma FRAP of around 4% at 40 minutes after ingestion: mean increase was 44 ± 9 (SE) μmol/l. Excretion of polyphenolic antioxidants was also fast, peaking at 60–90 minutes, with significant correlation between urinary FRAP values and urinary total phenolic concentrations* ($r = 0.845$, $p < 0.001$). *In control studies, no increase in plasma or urine FRAP values was seen after intake of water. Although the amount of antioxidants absorbed was relatively small and the increase in plasma antioxidant power was of short duration, results demonstrate that some potentially anticarcinogenic polyphenolic antioxidants in green tea enter the systemic circulation soon after ingestion and cause a significant increase in plasma antioxidant status. This increase may, in turn, lower oxidative damage to DNA and so decrease risk of cancer.*

Introduction

Tea, from the leaves of *Camellia sinensis*, is consumed in large amounts throughout the world (1), although the form and quantity in which tea is taken vary in different geographical areas and among ethnic groups (1,2). Tea, particularly green tea, is rich in polyphenolic flavonoid compounds with antioxidant properties, and these may inhibit oxidative damage to important biomolecules, including lipoproteins and DNA (3–10). Oxidative damage to DNA is associated with increased risk of cancer (10,11), and there is evidence that improved antioxidant status *in vivo*, achieved, e.g., by dietary supplementation, lowers oxidative damage to DNA and may help lower cancer risk (10,12). Animal studies have shown that green tea antioxidants can protect against powerful mutagens (13–20), and some epidemiological studies have reported a lower incidence of cancer in association with high intake of green tea (21–24). A clearly protective role for tea antioxidants, however, has not been established (2,8,25–27).

However powerful the *in vitro* anticarcinogenic activity of tea antioxidants may be, potential health benefits are unlikely to be realized if tea antioxidants are inactivated in the gut or are not absorbed. Absorption studies in human subjects are few and show conflicting results (28–30). In studies assessing absorption and systemic distribution of tea antioxidants, postingestion response in the "total antioxidant power" of plasma can be used as a marker of absorption, the rationale being that if antioxidants are absorbed with their activity conserved, the antioxidant power of plasma will increase. Results of the two small tea antioxidant studies of this type (31,32), however, show no agreement: no detectable postingestion change in the total antioxidant power of plasma was reported by one group (31), and the other (32) reported average increases of 40–50%, and approaching 100% in some subjects, within one hour. These studies used different methods of assessing total antioxidant power, and a combination of methodological differences, insufficient dosing with tea antioxidants, different sampling times, and possible effects of storage on antioxidants in plasma before analysis may help account for the conflicting results. Methodological differences notwithstanding, however, it is difficult to reconcile a near doubling of plasma antioxidant power in one study with an undetectable response in the other. It is not yet clear, therefore, whether antioxidant compounds in green or black tea are absorbed in significant amounts after ingestion. Further study into the bioavailability and potential beneficial effects of tea antioxidants is clearly needed.

In this study, changes in antioxidant power were monitored, in plasma and urine, at timed intervals after ingestion of a known amount of green tea. The marker of antioxidant

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absorption was the ferric reducing/antioxidant power (FRAP) value (33,34) (US patent pending), which enables sensitive and reproducible results to be obtained on complex biological samples and is simple and rapid enough to be performed on freshly collected specimens. Owing to its speed and high sensitivity, exactly timed, serial samples can be measured and small postingestion differences can be detected. This makes the FRAP assay a useful antioxidant bioavailability tool. In addition to monitoring postingestion changes in plasma and urinary antioxidant power, urinary excretion of total phenolics was also measured and correlated with urinary excretion of antioxidant power after ingestion of green tea.

Materials and Methods

Subjects

A total of 12 healthy adults consented to take part in the study; however, owing to difficulty in obtaining serial blood samples from one subject and gastric intolerance of the tea preparation in another, results are presented for 10 subjects (5 men and 5 women).

Test Methods

The FRAP assay was performed, as previously described in detail (33,34), using a Cobas Fara centrifugal analyzer (Roche Diagnostics, Basel, Switzerland). The FRAP assay is a rapid, automated method that measures the combined reductive (antioxidant) power of electron-donating antioxidants within the test sample and employs a timed, redox-linked ferric/ferrous tripyridyltriazine colorimetric reaction. The FRAP assay has a limit of detection of <2 μmol/l reducing/antioxidant power, and precision is excellent; within- and between-run coefficients of variation (CVs) are <0.5% and <1.0%, respectively, at 500–2,000 μmol/l ($n > 8$ in each case). Total phenolics in urine were measured using the Folin-Ciocalteu reaction (28,35). Creatinine was measured in plasma and urine with an alkaline picrate test kit (Roche) on a Cobas Fara analyzer. FRAP assay results were expressed as micromoles per liter of antioxidant power for plasma and as micromoles of antioxidant power per micromole of creatinine for urine. Total phenolics (μmol) in urine were also reported per micromole of creatinine.

Preparation of Tea

Strong green tea was freshly prepared on each occasion by adding 500 ml of boiling distilled water to 20 g of dry tea leaves ("China green tea," Shanghai Tea Import and Export) purchased from a local shop; the tea was allowed to infuse for 10 minutes and filtered, and the final volume was adjusted to 400 ml with distilled water. The FRAP value of a small aliquot of each tea infusion, as drunk, was measured within three hours of preparation.

Protocol and Samples

Subjects (≤3 on any 1 morning) were asked to arrive fasting. Informed consent was obtained, then a heparinized blood sample and a urine sample were collected from each subject; 400 ml of tea, or as much as each subject could comfortably drink, which in 2 of the 10 subjects was around 300 ml, were ingested over the next 10 minutes. Additional venous blood samples were collected into heparinized blood collection tubes at 20, 40, 60, and 120 minutes after ingestion. Blood samples were kept chilled and in the dark until separation of plasma from the erythrocytes, which was within 2.5 hours of blood collection. The plasma total antioxidant/reducing power, as FRAP, was measured in triplicate immediately thereafter. Urine samples were collected at 30-minute intervals without preservative into clean glass containers for up to three hours after ingestion. The urine FRAP values were measured, after appropriate dilution in distilled water, within four hours of collection. Subjects remained fasting, except for the initial ingestion of tea and subsequent sips of distilled water, for the entire period of sample collection. At least four weeks later, 7 of the 10 subjects (5 men and 2 women) repeated the study, drinking warm distilled water in place of tea. Ethical approval for this study was granted by the Ethics Subcommittee of the Hong Kong Polytechnic University, and all procedures involving human subjects complied with the Declaration of Helsinki, as revised in 1989.

Although it was planned that all volunteers would take the same fixed dose of tea antioxidants, two volunteers found the strong, bitter tea distasteful and drank around 300 ml; the other eight subjects drank 400 ml. The doses, therefore, were not standardized to body mass index but were known: mean doses of tea and antioxidant power for the 10 subjects were 375 ± 53 (SD) ml and 19,809 ± 7,106 (SD) μmol, respectively. Six of the 10 subjects collected all urine passed at each 30-minute interval over three hours; four subjects retained only aliquots at each time period.

For analysis of results, the following were calculated: 1) peak increase in FRAP after tea ingestion over fasting plasma levels, 2) area under the curve (AUC, by the trapezoid method) for increase in plasma FRAP over two hours, 3) correlation of AUC with dose of antioxidants ingested, 4) correlation of baseline plasma FRAP level and response, as AUC, 5) increase in urinary FRAP after ingestion of tea, and 6) correlation between phenolics and FRAP in urine after ingestion of tea.

Statistical Analysis

Repeated-measures analysis of variance was used to investigate timed postingestion changes in plasma FRAP values (n = 10). The paired Wilcoxon signed rank test was used to compare changes in plasma FRAP values after ingestion of tea and water and to compare baseline (fasting)

plasma FRAP values before ingestion of tea and of water ($n = 7$).

Results

Absorption of polyphenolic antioxidants from green tea was fast, resulting in a significant ($p < 0.001$) increase in the FRAP value of plasma. The peak increase occurred at 20–40 minutes after ingestion, and the mean 40-minute increase was 44 ± 9 (SE) μmol/l (Figure 1) or around 4%. The magnitude of response was not related to the dose of tea antioxidants ingested or the baseline FRAP value (results not shown). The increase in plasma FRAP was of short duration, and values returned to or approached baseline (fasting) levels by two hours after ingestion in most, but not all, subjects. Plasma creatinine concentrations did not change during the course of the study, indicating no confounding net changes in the plasma water content owing to ingestion of liquid. In subjects who repeated the test with water, fasting plasma FRAP values were not significantly different from the previous occasion [1,156 ± 65 and 1,169 ± 53 (SE) μmol/l], and no postingestion increases in plasma FRAP were seen (Figure 1).

The AUC was calculated on the basis of increases in plasma FRAP from baseline up to two hours after ingestion of tea. There was no direct correlation between dose of tea antioxidants ingested and AUC between subjects; nor was any significant correlation seen between dose and the 40-minute (peak) increase in plasma FRAP.

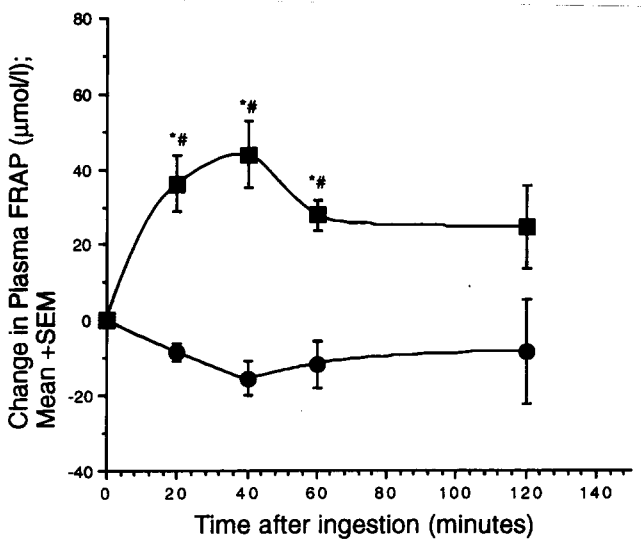

Figure 1. Change in plasma antioxidant/reducing power, expressed as ferric reducing/antioxidant power (FRAP), after intake of green tea (*filled squares*, $n = 10$) or water (*filled circles*, $n = 7$). There was a rapid and significant response to tea, peaking at 40 min after ingestion. *, Significantly different from baseline (fasting) value ($p < 0.001$, by repeated-measures analysis of variance test). #, Significantly different from corresponding paired values after ingestion of water ($p < 0.02$, by Wilcoxon signed rank test). No significant changes in plasma FRAP values were seen after ingestion of water.

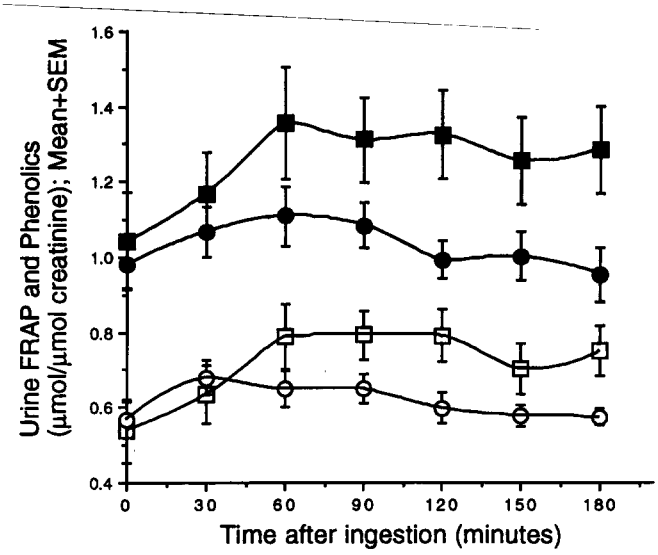

Figure 2. Urinary excretion of antioxidant/reducing power, expressed as FRAP (*filled symbols*), and phenolics (*open symbols*) after intake of green tea (*squares*, $n = 10$) and water (*circles*, $n = 7$). FRAP and phenolic excretion increased after ingestion of green tea; this pattern was not seen after ingestion of water.

The increase in urinary excretion of antioxidant power was fairly rapid, with peak excretion at 60–90 minutes after ingestion. This was also the time of peak total excretion of phenolics (Figure 2). Furthermore, after tea, but not after water, there was a significant correlation between urine antioxidant power and total phenolic concentration ($r = 0.845$, $p < 0.001$) (Figure 3). This indicates that the increase in antioxidant power of urine was related to excretion of the absorbed green tea polyphenolic antioxidants. There was no apparent relationship between plasma response, in terms of AUC over two hours, and the total amount of antioxidant power excreted (as μmol FRAP).

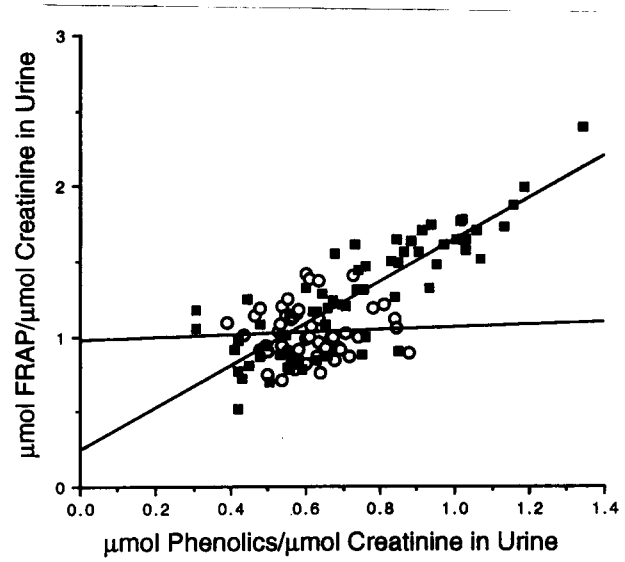

Figure 3. Relationship between urinary phenolics and antioxidant/reducing power, expressed as FRAP, before and after intake of tea (*squares*) and water (*circles*). There was a significant, direct correlation between urinary phenolics and FRAP after ingestion of tea ($r = 0.845$, $p < 0.001$); no such relationship was seen after water intake.

Discussion

Human studies of absorption of polyphenolic antioxidants in tea have been few, and their results have been conflicting (27–32). In the present study, results demonstrated a clear and rapid "spike" of antioxidant power into the plasma after ingestion of green tea, indicating that at least some of the antioxidants in green tea are absorbed and reach the systemic circulation. Excretion of absorbed antioxidants also appeared to be rapid, mirroring excretion of phenolic compounds in the urine.

The speed (peak at 20–40 min) and magnitude (average increase 4%) of response seen in the present study could account for the apparent lack of response reported in a previous study, in which black tea was taken (31). Blood samples were collected between one and three hours after ingestion, thereby missing the peak tea-related increase in plasma antioxidant power seen in this study, and the very small increases remaining one hour after ingestion may well have been undetectable by the method used.

In this study, peak responses in all subjects were similar, and plasma FRAP values approached baseline by two hours after ingestion in most subjects. Two subjects showed a prolonged response, however, indicating that total absorption and metabolism of tea antioxidants may differ between individuals. The difference may be related to prolonged absorption and/or differential metabolism or elimination in some individuals. This could help account for the varying epidemiological findings (2,25,36). Individual, perhaps genetically modulated (37), response to tea consumption deserves further study. No correlation was seen between dose and plasma response in the present study, possibly indicating that the absorption mechanism was saturated at even the lowest dose of tea ingested and/or that absorption of antioxidants in tea is selective. In a small ($n = 4$) study (38) of absorption of polyphenols from green tea, measured plasma levels of individual catechins at one hour after ingestion covered a very wide range. Furthermore, peak concentrations of catechins in plasma and urine have been reported to occur at between one and four hours and three and six hours after ingestion, respectively (38–40). This implies that the rapid postingestion increase in antioxidant power seen in the present study, which peaked at 20–40 minutes, may have been mediated by non-catechin phenolic antioxidants, and this deserves further study.

Results of this study showed a plasma AUC of 61.6 ± 14.4 (SE) μmol/l/h in the two hours after ingestion of green tea. This indicates a rather poor systemic availability of antioxidants from tea but supports the rapid response seen in the single published study (32) that reported a detectable postingestion response. The mean increase in plasma antioxidant power after one cup (300 ml) of tea was, however, surprisingly large (40%; $n = 5$) in that previous study (32). Furthermore, although the distribution of the published data is not clear, interindividual differences in response appear to be very wide (32). Such large variation is difficult to explain but may be related to individual differences in absorption or elimination, as suggested above. Nevertheless, although individual and methodological differences may have contributed to the differences in magnitude and variation of response seen, results of the present and previous (32) study indicate that absorption and systemic distribution of ingested tea antioxidants are rapid.

Results of this study indicate that at least some of the antioxidants in green tea are absorbed and systemically distributed. Regular consumption of green tea could, therefore, improve antioxidant defense and lower cancer risk. Furthermore, tea antioxidants left unabsorbed in the gut may act to conserve other dietary antioxidants and could confer local protection. Whether absorption of tea antioxidants is of significant benefit to health remains to be established, however. Intake of black, as opposed to green, tea is high throughout the United Kingdom and Ireland, but cancer has clearly not been eradicated in these areas. Epidemiological studies, however, have no power to discriminate or reveal differences if rates of disease and/or the population means of the agent under investigation do not vary significantly between the populations being studied (41). In addition, variables such as socioeconomic status, smoking, and dietary habits combine to confound results, and consistent dose-related effects of black tea have yet to emerge (36,42). It has been suggested that taking tea with milk, which is the preferred way in which black tea is taken in the United Kingdom and Ireland, may affect absorption and/or antioxidant action of tea antioxidants (32,42). However, milk does not appear to affect the absorption of catechins (39). Further study of factors affecting absorption of tea antioxidants, including characterization of the individual antioxidant(s) responsible for the rapid postingestion increase in plasma antioxidant power seen in this study, is clearly needed.

In summary, results of this study confirm that polyphenolic antioxidant compounds in green tea are absorbed and enter the systemic circulation rapidly after ingestion and that their absorption causes a significant increase in plasma antioxidant power. Further study is needed to establish whether regular ingestion of green tea leads to a significant response in terms of decreased oxidative damage to DNA and, thereby, to lowered cancer risk in humans, to investigate which antioxidant(s) in green tea is bioavailable, and to investigate whether some individuals are more responsive to green tea antioxidants than others.

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