Can black tea influence plasma total homocysteine concentrations?[1–3]

Jonathan M Hodgson, Valerie Burke, Lawrence J Beilin, Kevin D Croft, and Ian B Puddey

ABSTRACT

Background: Polyphenols can act as acceptors of methyl groups during the metabolism of methionine to homocysteine. This may result in elevations in plasma total homocysteine (tHcy) concentrations after ingestion of polyphenol-rich beverages such as tea.

Objectives: Our major objective was to determine whether regular, moderate-to-high intakes of black tea alter tHcy concentrations. We also assessed the relation between the degree of *O*-methylation of tea-derived polyphenols and the change in tHcy with regular ingestion of tea.

Design: Twenty-two subjects completed a randomized, controlled crossover study. Subjects consumed 1250 mL black tea/d (5 cups each containing 2 g tea leaves in 250 mL boiled water) and 1250 mL hot water/d for 4 wk each. Fasting tHcy concentrations and 24-h urinary excretion of 4-*O*-methylgallic acid (4OMGA, the major *O*-methylated metabolite of gallic acid) were measured at the end of each period. 4OMGA was used as a marker of overall *O*-methylation of tea-derived polyphenols.

Results: Black tea did not significantly alter mean (±SEM) tHcy concentrations (9.9 ± 0.5 and 10.0 ± 0.5 μmol/L for the hot water and black tea periods, respectively). However, the increased excretion of 4OMGA as a consequence of black tea consumption was positively associated with the change in tHcy from the hot water period to the black tea period ($r = 0.55$, $P = 0.008$). Subjects in the bottom quartile of increase in 4OMGA excretion had a significant decrease in tHcy (−0.28 ± 0.10 μmol/L; $P = 0.046$), and those in the top quartile had a significant increase in tHcy (0.78 ± 0.16 μmol/L; $P = 0.005$).

Conclusions: Overall, regular ingestion of black tea did not alter mean tHcy concentrations. However, individual differences in *O*-methylation of polyphenolic compounds may influence the ultimate effects of black tea on tHcy. *Am J Clin Nutr* 2003;77:907–11.

KEY WORDS Black tea, total homocysteine, polyphenols, *O*-methylation, urinary 4-*O*-methylgallic acid

INTRODUCTION

Elevated plasma total homocysteine (tHcy) concentrations are associated with an increased risk of atherothrombotic cardiovascular disease. This association is independent of other risk factors and is dose related, and there is increasing evidence that it is causal (1). Homocysteine is an amino acid derived from the demethylation of dietary methionine. The metabolism of homocysteine is influenced by several dietary factors, including folate, vitamin B-12, vitamin B-6, and betaine (2), and may be influenced by the intake of polyhydroxylated phenolic compounds (polyphenols) (3; **Figure 1**).

Dietary polyphenols were found to increase tHcy concentrations (3). Some of the richest dietary sources of polyphenols are beverages, including tea and coffee. Coffee is one of the main dietary factors implicated in increasing tHcy concentrations. Population studies showed that coffee is associated with elevated tHcy concentrations (4–10), and in controlled intervention studies, regular, chronic ingestion of coffee resulted in clinically relevant increases in tHcy concentrations (11, 12). The effect of coffee to increase tHcy concentrations is predominantly due to chlorogenic acid (3), which is a phenolic acid and the major polyphenol present in coffee. A controlled intervention study showed that very high doses of tea also increase tHcy concentrations (3). This may be due to polyphenols present in tea, including gallic acid, which is the major phenolic acid present in tea. However, results of cross-sectional population studies do not support a tHcy-increasing effect of tea. These studies generally showed inverse associations of tea intake with tHcy (4–7), which were attenuated after adjustment for coffee intake and other potential confounders (5–7). The effect on tHcy of a dose of tea that is more representative of ordinary population intakes has not been investigated in an intervention study.

It has been suggested that dietary polyphenols may contribute to an elevation in tHcy concentrations by acting as acceptors of methyl groups (3; Figure 1). If dietary polyphenols can alter tHcy concentrations, then the overall effects of a polyphenol-rich beverage may relate to polyphenol metabolism. Individual differences in the degree of *O*-methylation of polyphenols may influence tHcy concentrations. If this hypothesis is correct, then a greater degree of *O*-methylation of a specific dose of polyphenols should be associated with higher tHcy concentrations.

Our aim in the present study was to determine whether regular ingestion of black tea, at an intake that is in the moderate to high range in tea-drinking populations, alters tHcy concentrations. To investigate whether polyphenol metabolism is an important factor influencing tHcy concentrations, the relation between the *O*-methylation of gallic acid and the effects of black tea on tHcy concentrations were also investigated.

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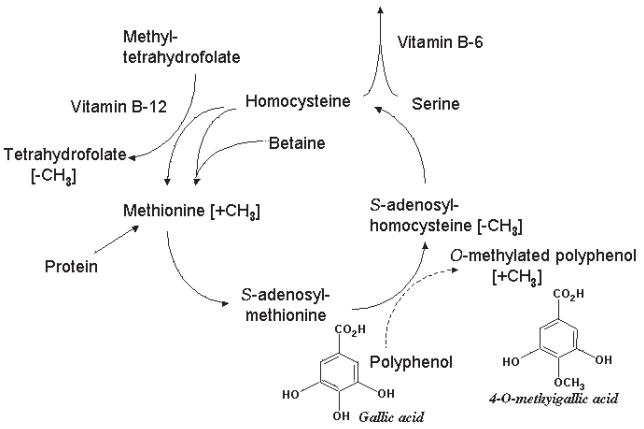

FIGURE 1. Hepatic metabolism of homocysteine and proposed interaction with *O*-methylation of polyphenols.

SUBJECTS AND METHODS

Subjects

Twenty-two subjects (16 men and 6 women) were recruited from the general population. Subjects were excluded if they were premenopausal women, if they were postmenopausal women receiving hormone replacement therapy, if their usual alcohol intake exceeded 4 standard drinks/d (>40 g alcohol/d), if they were current smokers or were former smokers who had stopped smoking for <6 mo, if they were taking any medication, if their body mass index (in kg/m^2) was >35, or if they had any history of heart, liver, renal, or gastrointestinal diseases or disorders or of diabetes. The Royal Perth Hospital Ethics Committee approved the project, and all participants gave written informed consent.

Study design

The study was a randomized, controlled crossover trial with 4-wk intervention periods preceded by a 4-wk baseline period (13). During the 12 wk of the study, subjects ceased intake of caffeine-containing beverages (except those assigned), including tea, coffee, chocolate drinks, and cola, and of herbal teas. During baseline all subjects drank 1250 mL hot water/d (5 cups each containing 250 mL hot water). Subjects were then randomly assigned with the use of computer-generated random numbers to consume 1250 mL black tea/d (5 cups each containing 2 g tea leaves in 250 mL boiled water) or to continue to consume 1250 mL hot water/d for 4 wk; the subjects then consumed the alternate drink for an additional 4 wk.

All measurements were performed at baseline and at the end of the 2 intervention periods. Blood samples were taken in the morning after the subjects had fasted overnight. Samples were prepared by using standard procedures and were then frozen at −80 °C until assayed. A 24-h urine sample was collected at the end of the baseline period and at the end of each of the intervention periods. Urine samples were frozen at −80 °C until assayed.

A "world blend" leaf tea (a blended black tea) was obtained from the Tea Trade Health Research Association (Toronto). To standardize each cup of tea as far as possible, instruction on the method of tea preparation was given to all subjects. A supply (500 g) of leaf tea was provided to each subject. Two grams of tea leaves placed into a spring-handled infuser was infused in 250 mL boiled water for 1 min with constant movement; the tea was then consumed without additives, including milk and sugar. Compliance was measured by using the total weight of tea leaves consumed. Five cups of black tea per day, each containing 2 g tea leaves, equates to 280 g over 4 wk. Mean (± SEM) tea-leaf use was 293 ± 11 g. The control drink was the same volume of boiled water consumed hot. To avoid the possibility of acute effects, we instructed the subjects not to drink tea in the morning on days when blood samples were to be collected.

Participants were instructed not to make any changes to their usual food intake, alcohol consumption, or physical activity during the study. Usual food intake, alcohol consumption, and physical activity were assessed by questionnaire at baseline and were monitored by a dietitian throughout the intervention to ensure minimal changes throughout the study. Body weight and height were measured and body mass index was calculated both at baseline and at the end of each intervention period.

Biochemistry

Plasma total L-homocysteine concentrations were measured by using a Fluorescence Polarization Immunoassay on an Abbott IMx analyzer (Abbott Laboratories, Abbott Park, IL). The tHcy value included free monomeric homocysteine, free dimeric homocysteine, protein-bound forms, and mixed dimeric low-molecular-mass forms (14). The interassay CV for homocysteine measurement was <5%. Serum folate concentrations were measured by using ion capture on an Abbott AxSYM analyzer (Abbott Laboratories) (15). The interassay CV for folate measurement was <12%. Vitamin B-12 concentrations were measured by using a microparticle enzyme intrinsic factor assay on an Abbott AxSYM analyzer (16). The interassay CV for vitamin B-12 measurement was <15%. Vitamin B-6 was measured in serum as pyridoxal by using a microbiological assay featuring *Lactobacillus casei*. The major form of vitamin B-6 in serum is pyridoxal-5-phosphate. Because *L. casei* is sensitive only to pyridoxal, the sample was first dephosphorylated by using trichloroacetic acid and heat. Concentrations were measured on the basis of optical density at 640 nm (17, 18).

Urinary concentrations of 4-*O*-methylgallic acid (4OMGA), the major *O*-methylated metabolite of gallic acid in humans (19), were used as a marker of the overall *O*-methylation of black tea–derived polyphenols. 4OMGA was measured in 24-h urine samples at the end of the baseline period and at the end of each of the intervention periods by using a previously described method (20, 21). Briefly, urine (2 mL) and 2-hydroxy-3-naphthoic acid (1 μg, internal standard) were acidified to pH 4.8 with dilute hydrochloric acid. Thirty microliters of β-glucuronidase (EC 3.2.1.31) with 3000 units of activity (catalog number G707; Sigma Chemical Co, St Louis) was added to the urine samples, and after the samples were mixed, they were placed in a water bath at 37 °C for 24 h and were mixed occasionally. Samples were extracted with ethyl acetate (2 mL) and then centrifuged at 2000 × *g* and 4 °C for 10 min. The organic phase was dried under nitrogen and then derivatized with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (50 μL) and pyridine (50 μL) at 40 °C for 30 min. The 4OMGA esters were analyzed on a Hewlett-Packard HP 5890 gas chromatograph coupled to an HP 5970 mass spectrometer fitted with an HP-1 cross-linked methyl silicone column (25 m × 0.20 mm, 0.33-mm film thickness; Hewlett-Packard, Palo Alto, CA); the carrier gas was helium. An inlet pressure of 30 kPa was used, and

TABLE 1
Biochemical measures at baseline and at the end of 4 wk of regular ingestion of hot water or black tea[1]

	Baseline	Hot water	Black tea
Serum total cholesterol (mmol/L)	5.8 ± 0.1	5.8 ± 0.1	5.7 ± 0.1
Serum triacylglycerols (mmol/L)	1.1 ± 0.1	1.0 ± 0.1	1.1 ± 0.1
Systolic blood pressure (mm Hg)	125 ± 5	121 ± 3	120 ± 3
Diastolic blood pressure (mm Hg)	74 ± 2	72 ± 2	72 ± 2
Plasma total homocysteine (μmol/L)	10.2 ± 0.5	9.9 ± 0.5	10.0 ± 0.5
Serum folate (nmol/L)	22.8 ± 1.9	23.1 ± 1.4	23.0 ± 1.3
Serum vitamin B-6 (nmol/L)	47.8 ± 3.0	44.8 ± 3.3	43.5 ± 3.1
Serum vitamin B-12 (pmol/L)	239 ± 19	237 ± 21	246 ± 23
Urinary 4-*O*-methylgallic acid (nmol/mmol creatinine)	28 ± 4	39 ± 9	548 ± 65[2]

[1] $\bar{x}$ ± SEM; $n = 22$. A randomized, controlled crossover trial. During the intervention, subjects consumed 1250 mL black tea/d (5 cups each containing 2 g tea leaves in 250 mL boiled water) and 1250 mL hot water/d for 4 wk each.
[2] Significantly different from hot water, $P < 0.001$ (paired t test).

injections were made in a splitless mode. The initial column temperature of 120 °C was held for 0.5 min, and then the temperature was programmed to increase 15 °C/min to 280 °C, which was held for 5 min. The mass spectrometer was operated in the electron impact mode (70 eV). The major characteristic ion for the 4OMGA trimethylsilyl derivative (mass-to-charge ratio = 370) and the molecular ion minus a methyl group [for identification of the internal standard (mass-to-charge ratio of the molecular ion − 15 = 317)] were monitored in the selected ion monitoring mode. Peak identification was made by comparing retention times and mass spectra with those of authentic standards. The authentic standard of 4OMGA was prepared according to a previously published procedure (22). For quantification, calibration curves were established by measuring peak areas for given concentrations of 4OMGA and internal standard to obtain a response factor. The intraassay CV was 5%.

Statistical analyses

Statistical analyses were performed by using SPSS 11.0 software (SPSS Inc, Chicago). Results are presented as means ± SEMs, and $P < 0.05$ was the level of significance. The paired samples t test was used to determine the significance of any effects of black tea, including changes in tHcy concentration within quartiles of increase in urinary excretion of 4OMGA. Pearson's correlation coefficient (r) with two-tailed P value was used to determine the degree and direction of association between variables. Linear regression was used to determine the relation between changes in tHcy concentration and changes in urinary excretion of 4OMGA after adjustment for other variables. Analysis of variance was used to determine whether consumption of black tea led to higher tHcy concentrations in subjects in whom more gallic acid was methylated to 4OMGA than in subjects in whom less gallic acid was methylated to 4OMGA. Group differences were analyzed by using Tukey's adjustment.

RESULTS

Baseline biochemical measures are shown in **Table 1**. The subjects were aged between 43 and 75 y ($\bar{x}$ = 59 ± 1.6 y) and had a mean body mass index of 27.0 ± 0.6. They had mildly elevated serum total cholesterol concentrations and were normotensive. Plasma tHcy concentrations and serum folate, vitamin B-6, and vitamin B-12 concentrations were all within the normal range. At baseline, tHcy was significantly negatively correlated with folate ($r = -0.61$, $P = 0.003$) and vitamin B-12 ($r = -0.47$, $P = 0.03$) but was not significantly associated with vitamin B-6, serum lipids, or blood pressure.

Body mass index, serum lipid concentrations, and blood pressure were unchanged during the intervention. Ingestion of 5 cups black tea/d for 4 wk did not alter tHcy, folate, vitamin B-12, or vitamin B-6 concentrations (Table 1). Although tHcy concentrations were unchanged during the intervention, the tHcy response to ingestion of black tea varied between subjects. In comparison with the ingestion of hot water, the ingestion of black tea significantly increased urinary 4OMGA excretion (Table 1). The ingestion of black tea increased 4OMGA excretion in all subjects, but there was great variability between the subjects in their 4OMGA response.

The increased excretion of 4OMGA as a consequence of black tea consumption, as assessed by the change in 4OMGA excretion from the hot water period to the black tea period, was significantly positively associated with the change in tHcy concentration from the hot water period to the black tea period (r = 0.55, P = 0.008). This association remained significant after adjustment for age, sex, body mass index, and baseline folate, vitamin B-12, and vitamin B-6 concentrations. Subjects in the bottom quartile of increase in 4OMGA excretion had significant decreases in tHcy concentrations (−0.28 ± 0.10 μmol/L; P = 0.046). Subjects in the top quartile of increase in 4OMGA excretion had significant increases in tHcy concentrations (0.78 ± 0.16 μmol/L; P = 0.005). In addition, there was a significant difference in tHcy concentrations after ingestion of black tea between the subjects in the top and bottom quartiles of increase in 4OMGA excretion (**Figure 2**).

The serum folate, vitamin B-12, and vitamin B-6 concentrations at baseline were not significantly correlated with changes in tHcy concentrations between the periods. In addition, there were no significant relations between changes in folate, vitamin B-12, and vitamin B-6 concentrations and changes in tHcy concentrations between the periods.

DISCUSSION

We showed that a regular, moderate-to-high intake of black tea did not alter mean tHcy concentrations. However, the increased excretion of 4OMGA as a consequence of consuming 5 cups black tea/d was associated with the change in tHcy concentration. Subjects in whom more gallic acid was methylated to 4OMGA had

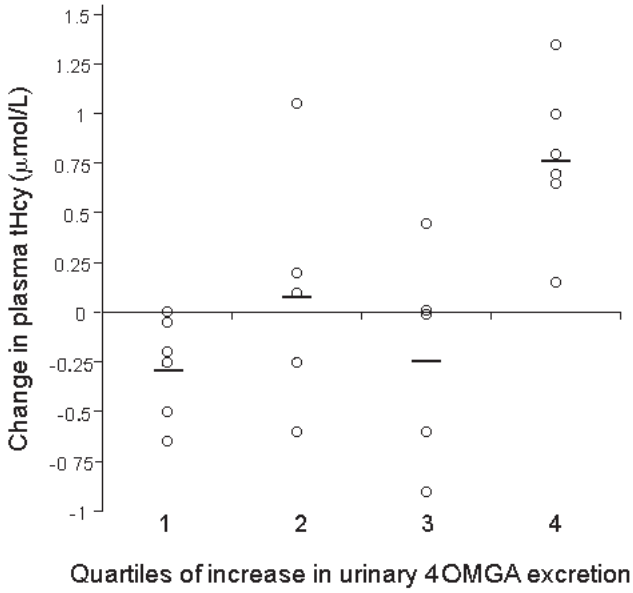

FIGURE 2. Relation between individual (○) and mean (—) increases in 24-h urinary 4-*O*-methylgallic acid (4OMGA) excretion by quartile from the hot water period to the black tea period and changes in fasting plasma total homocysteine (tHcy) concentrations in subjects consuming 1250 mL black tea/d and 1250 mL hot water/d for 4 wk each. The mean value for the highest quartile was significantly different from that for the lowest quartile, $P = 0.005$ (one-way ANOVA with Tukey's adjustment for multiple comparisons).

significant increases in tHcy concentrations, and those in whom less gallic acid was methylated to 4OMGA had significant decreases in tHcy concentrations. These results are consistent with the hypothesis that dietary polyphenols act as acceptors of methyl groups (Figure 1) and contribute to increases in tHcy concentrations. They are also consistent with a suggested potential tHcy-lowering effect of tea (4–7).

The term *tea* refers to the leaves of the plant *Camellia sinensis* and the infusions derived from them. Tea is the most popular beverage worldwide, apart from water, and therefore any effects on cardiovascular disease risk may apply to large populations. The 2 main types of tea are green and black; both are rich in polyphenolic compounds and contain caffeine. Black tea, the more popular beverage, is produced by promoting the enzymatic oxidation of flavonoids in the tea leaf, producing a range of flavonoids, many of which are yet to be characterized. One cup of black tea provides ≈150–300 mg polyphenols, of which ≈90% are flavonoids such as theaflavins and thearubigins (many of which include gallate esters) and ≈10% are free gallic acid (21, 23). Thus, consumption of black tea could represent a major portion of the total intake of flavonoids and gallic acid. Many of these polyphenols may be *O*-methylated by the enzyme catechol *O*-methyltransferase (EC 2.1.1.6; 23–25).

The product of gallic acid *O*-methylation, 4OMGA, is likely to provide the best available indicator of the overall *O*-methylation of tea-derived polyphenols. We previously showed that 4OMGA is an important metabolite of black tea polyphenols (21) and is a marker of black tea intake in humans (19). We identified several compounds derived from black tea, but only the *O*-methylated gallic acid increased consistently after drinking black tea (19). That is, measurement of 4OMGA had the least amount of interference from the background diet. Gallic acid and gallate esters can be derived from dietary sources other than tea. However, in the present study, background 4OMGA excretion was low, and ingestion of black tea (5 cups/d) increased urinary 4OMGA excretion ≥3-fold and, on average, 14-fold (Table 1). In addition, black tea contains a complex mixture of polyphenols, many of which have been difficult to characterize (26). Even if specific *O*-methylated flavonoids derived from black tea can be identified and measured, they may not provide a better indication of overall *O*-methylation of black tea polyphenols than that provided by 4OMGA.

The best available evidence for an effect of black tea on cardiovascular disease comes from prospective cohort studies. The polyphenolic compounds found in tea are suggested to be the main components responsible for any benefit on cardiovascular disease risk (27–29). Studies showing that both black tea intake and flavonoid intake are inversely associated with the risk of cardiovascular disease (28, 29) are consistent with benefits of polyphenols derived from tea. Beneficial effects of tea-derived flavonoids on vascular function, an early indicator of vascular disease, may partially account for this association (30, 31). Although the weight of evidence suggests that higher tea consumption reduces the risk of cardiovascular disease, this has not been an entirely consistent finding in population studies (32). Studies conducted in the United Kingdom suggest a higher risk of cardiovascular disease with higher black tea intake (32). This may be due to a confounding effect of socioeconomic status and related variables, but other variables may be involved.

Individual differences in *O*-methylation of polyphenols may be associated with the level of benefit or detriment of tea on cardiovascular disease risk through effects on homocysteine (Figure 1) and vascular function. There is evidence that elevations in tHcy concentrations contribute to an increased risk of cardiovascular disease via impairment in vascular function (33). We previously reported that regular ingestion of tea improves vascular function (30). Consistent with the findings in the present study for changes in tHcy concentrations, the degree of improvement in vascular function was negatively associated with the increased excretion of 4OMGA as a consequence of black tea consumption ($r = -0.79$, $P = 0.007$) (JM Hodgson, IB Puddey, V Burke, GF Watts, and LJ Beilin, unpublished observations, 2002). That is, improvements in vascular function were greatest in the subjects in whom gallic acid was methylated the least. These results provide evidence for a possible link between polyphenol metabolism and cardiovascular disease via effects on homocysteine and vascular function.

Despite the potential of polyphenols to increase tHcy concentrations in some persons, results of cross-sectional population studies do not generally support a homocysteine-increasing effect. Indeed, these studies overall suggest more of an inverse relation between tea intake and tHcy concentrations (4–7). Other components of tea and the background diet may therefore be important modifiers of any effects of tea on tHcy concentrations and vascular function. Low dietary intakes of the methyl donors folate, vitamin B-12, vitamin B-6, and betaine (Figure 1) may contribute to increased tHcy concentrations (2). The extent to which any effect of tea on homocysteine concentrations and vascular function may be related to the intake or status of these vitamins is uncertain. In addition, tea contains folate (≈5–10 μg/cup), which may offset any effect of gallic acid to increase tHcy concentrations or may contribute to

decrease tHcy concentrations (34). In the present study, serum concentrations of folate, vitamin B-12, and vitamin B-6 did not change because of the ingestion of tea. However, serum measurements may not adequately assess their status and their potential effect on homocysteine metabolism.

In conclusion, we showed that a regular, moderate-to-high intake of tea did not significantly alter tHcy concentrations. However, our results are consistent with the suggestion that dietary polyphenols can increase homocysteine concentrations by acting as acceptors of methyl groups and that individual differences in polyphenol metabolism affect tHcy concentrations after consumption of a polyphenol-rich beverage. This is the first study to show that differences between persons in polyphenol metabolism relate to the effects of a polyphenol-rich beverage on tHcy concentrations.

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JMH, VB, and IBP participated in conceiving, designing, and conducting the study; statistically analyzing the data; and writing the manuscript. LJB and KDC participated in conceiving and designing the study and writing the manuscript. None of the authors had any conflict of interest in connection with this article.

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