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Consumption of high doses of chlorogenic acid, present in coffee, or of black tea increases plasma total homocysteine concentrations in humans[1–3]

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ABSTRACT

Background: In population studies, high intakes of coffee are associated with raised concentrations of plasma homocysteine, a predictor of risk of cardiovascular disease. Chlorogenic acid is a major polyphenol in coffee; coffee drinkers consume up to 1 g chlorogenic acid/d.

Objective: We studied whether chlorogenic acid affects plasma total homocysteine concentrations in humans. For comparison we also studied the effects of black tea rich in polyphenols and of quercetin-3-rutinoside, a major flavonol in tea and apples.

Design: In this crossover study, 20 healthy men and women ingested 2 g (5.5 mmol) chlorogenic acid, 4 g black tea solids containing ≈4.3 mmol polyphenols and comparable to ≈2 L strong black tea, 440 mg (0.7 mmol) quercetin-3-rutinoside, or a placebo daily. Each subject received each of the 4 treatments for 7 d, in random order.

Results: Total homocysteine in plasma collected 4–5 h after supplement intake was 12% (1.2 μmol/L; 95% CI: 0.6, 1.7) higher after chlorogenic acid and 11% (1.1 μmol/L; 95% CI: 0.6, 1.5) higher after black tea than after placebo. Total homocysteine in fasting plasma collected 20 h after supplement intake was 4% (0.4 μmol/L; 95% CI: 0.0, 0.8) higher after chlorogenic acid and 5% (0.5 μmol/L; 95% CI: 0.0, 0.9) higher after black tea than after placebo. Quercetin-3-rutinoside did not significantly affect homocysteine concentrations.

Conclusions: Chlorogenic acid, a compound in coffee, and black tea raise total homocysteine concentrations in plasma. Chlorogenic acid could be partly responsible for the higher homocysteine concentrations observed in coffee drinkers. Whether these effects on homocysteine influence cardiovascular disease risk remains to be established. *Am J Clin Nutr* 2001;73:532–8.

KEY WORDS Polyphenol, coffee, tea, chlorogenic acid, quercetin-3-rutinoside, homocysteine, cardiovascular disease

INTRODUCTION

A high homocysteine concentration in blood is a risk factor for cardiovascular disease (1, 2). Epidemiologic studies suggest that coffee consumption might be one of the determinants of plasma homocysteine concentrations; plasma homocysteine concentrations in coffee drinkers are up to 2-μmol/L higher than those in coffee abstainers (3–5). The results of both an intervention study with unfiltered coffee (6) and an intervention study with filtered coffee (7) support the homocysteine-raising effects of coffee.

The compounds in coffee responsible for this effect are not known. The kahweol and cafestol present in only unfiltered coffee (8) are not responsible because both filtered and unfiltered coffee raise plasma homocysteine concentrations (6, 7). One candidate compound is chlorogenic acid, a polyphenol that occurs in large amounts in coffee but in only small amounts in other foods and beverages. Another candidate is caffeine. The amounts of chlorogenic acid and caffeine in coffee are comparable (9, 10). No studies have been done to investigate the effect of chlorogenic acid or caffeine on plasma homocysteine.

We thus studied the effect of chlorogenic acid on plasma homocysteine concentrations in healthy volunteers. An effect of chlorogenic acid on homocysteine might be mediated by metabolites of chlorogenic acid in the human body. Because metabolism of chlorogenic acid and other polyphenols is likely to occur via the same pathways, we also determined the effect on plasma homocysteine concentrations of polyphenols from black tea and of quercetin-3-rutinoside. Black tea is the tea most commonly consumed worldwide (11) and quercetin-3-rutinoside is a major flavonol in tea and apples (12, 13).

SUBJECTS AND METHODS

Subjects

Ten men and 10 women with a mean (±SD) age of 24 ± 8 y and body mass index (in kg/m^2) of 22.2 ± 2.5 participated. The

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TABLE 1
Change in plasma total homocysteine concentrations compared with placebo in postprandial and fasting plasma samples collected from 20 healthy volunteers after ingestion of chlorogenic acid, black tea, or quercetin-3-rutinoside for 7 d each[1]

Supplement	Intake of polyphenols	Change in total homocysteine concentration compared with placebo	
		Posprandial plasma	Fasting plasma
	mmol/d	*μmol/L*	
Chlorogenic acid	5.5	1.2 (0.6, 1.7)[2]	0.4 (0.0, 0.8)
Black tea	4.3	1.1 (0.6, 1.5)	0.5 (0.0, 0.9)
Quercetin-3-rutinoside	0.7	0.3 (−0.2, 0.7)	−0.0 (−0.4, 0.3)

[1] Mean (±SD) homocysteine concentrations after each supplement are shown in Figures 1–3. Postprandial blood samples were collected 4–5 h after the last supplement intake and fasting blood samples were collected ≈20 h after the last supplement intake.
[2] Mean (95% CI).

subjects were healthy as judged by a medical questionnaire; normal blood values for hemoglobin, hematocrit, and white blood cell counts; and absence of protein and glucose in urine. All subjects were nonsmokers. They were not allowed to take any drugs or other supplements during the study except for acetaminophen (paracetamol) and oral contraceptives. The study protocol was fully explained to the subjects and they gave their written, informed consent. The protocol was approved by the Medical Ethical Committee of the Division of Human Nutrition and Epidemiology.

Methods

Throughout the 4-wk study, subjects consumed a controlled diet low in polyphenols. To achieve this, we supplied the subjects daily with foods low in polyphenols that provided 90% of the energy required to maintain body weight. The remaining 10% of energy was chosen by the subjects from a list of food items low in polyphenols. Foods were considered low in polyphenols if they contained <15 mg quercetin or chlorogenic acid/kg; beverages were considered low in polyphenols if they contained <4 mg quercetin or chlorogenic acid/L (9, 14, 15). Because consumption of coffee and tea was not allowed, we provided the volunteers with the following substitutes: for coffee, an extract made of chicory, rye, and barley (Swiss coffee-like; Tayala AG, Birsfelden, Switzerland), and for tea, tea bags containing a mix of herbs (droommix; Piramide, Veenendaal, Netherlands) or tea bags containing stinging nettle (Jacob Hooy, Limmen, Netherlands). Chemical analyses indicated that these substitutes contained only minor amounts of catechins, flavonols, or chlorogenic acid.

In addition to the controlled diet, subjects ingested one of the following supplements each day: *1)* 2 g (5.5 mmol) chlorogenic acid (Fluka Chemie AG, Buchs, Switzerland), *2)* 4 g black tea solids (LN0173-02; kindly provided by Unilever Research Vlaardingen, Vlaardingen, Netherlands) with 30–40% polyphenols by weight (4 g black tea solids contains ≈4.3 mmol polyphenols, mainly catechins) (11), *3)* 440 mg (0.7 mmol) quercetin-3-rutinoside (Rutosidum DAB; BUFA BV, Uitgeest, Netherlands), or *4)* 0.5 g citric acid as a placebo (AC Citricum; Fagron, Nieuwerkerk A/D IJssel, Netherlands) (**Table 1**). The 2 g chlorogenic acid is comparable with the amount of chlorogenic acid in ≈1.5 L strong coffee, the 4 g black tea solids is comparable with ≈2 L strong black tea, and the amount of quercetin in 440 mg quercetin-3-rutinoside is comparable with the amount in ≈13 L black tea (15) and is 13 times higher than the average daily intake of quercetin (16). Subjects ingested each of these 4 supplements for 1 wk in random order. Before ingestion, the chlorogenic acid, quercetin-3-rutinoside, citric acid, and one-half of the black tea solids (2 g) were dissolved in hot water. Subjects consumed the supplements under our supervision just before the hot meal at noon. The other one-half of the black tea solids was used for tea preparation and consumption at home: 1 g between 0800 and 1000 (on Saturdays and Sundays, between 0800 and 1100) and 1 g between 1800 and 2000. The volunteers were urged to maintain their usual pattern of physical activity during the study.

On day 7 of each supplement period we collected 2 blood samples: 1 sample in the morning after subjects had fasted overnight, collected ≈20 h after the last supplement intake, and 1 postprandial sample, collected 4–5 h after the last supplement intake. We chose to take a fasting blood sample to exclude interference from food consumption shortly before blood sampling. We chose to collect the postprandial blood sample 4–5 h after supplement intake because the peak concentration of homocysteine after methionine loading, a precursor of homocysteine, occurs at this time point (17, 18). Because the black tea supplement was ingested 3 times daily, the blood sampling times after intake of the black tea supplement were as follows: the fasting sample in the morning was collected ≈12 h after the intake of the 1-g dose of black tea solids and ≈20 h after the intake of the 2-g dose on the previous day and the postprandial blood sample was collected 4–5 h after intake of the 2-g dose of black tea solids at 1200. Blood was collected into vacuum tubes (Venoject II; Terumo Europe NV, Leuven, Belgium) containing EDTA. Blood samples were immediately placed on ice and within 1 h were centrifuged at 2500 × *g* for 10 min at 4°C to obtain plasma. Plasma was separated and stored at −80°C. We also collected urine for the measurement of polyphenolic metabolites; these results will be published elsewhere.

Total homocysteine concentrations were measured by HPLC with fluorometric detection (19, 20). The interassay CV of the homocysteine assay was <8%. Folate and vitamin B-12 concentrations were measured with ion-capture IMx (Abbott Laboratories, Abbott Park, IL) (21, 22). The interassay CV of the folate assay was <12% and that of the vitamin B-12 assay was <7%. For the measurement of vitamin B-6 in the chlorogenic acid and placebo periods, we also collected fasting blood samples into vacuum tubes containing lithium-heparin and immediately stored the tubes at −80°C until analysis. The vitamin B-6 concentration was measured as pyridoxal-*P* (PLP) in EDTA-treated whole blood by HPLC (23) after precolumn derivatization with semicarbazide to obtain PLP-semicarbazone (24). The interassay CV of the vitamin B-6 assay was <7%.

We measured all postprandial blood samples in the same series of analyses. The fasting plasma samples for measure-

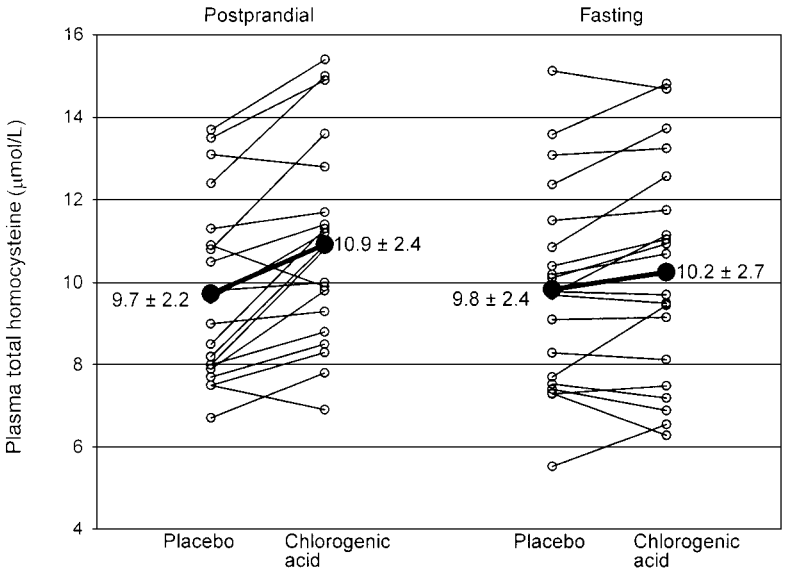

FIGURE 1. Plasma total homocysteine concentrations in 20 healthy subjects after they had consumed 2 g (5.5 mmol) chlorogenic acid or a placebo daily, for 7 d each, in random order. Postprandial blood samples were collected 4–5 h after the last supplement intake and fasting samples were collected ≈20 h after the last supplement intake. Shown are the individual (○) and mean ± SD (●) changes.

ment of total homocysteine, vitamin B-12, and folate concentrations were analyzed in 2 separate series of analyses on separate occasions: in the first series we measured the fasting plasma samples obtained after the placebo and chlorogenic acid periods and in the second series we measured the fasting plasma samples obtained after the placebo, black tea, and quercetin-3-rutinoside periods. Thus, a subject's plasma or blood samples obtained after treatment with chlorogenic acid, black tea, or quercetin-3-rutinoside were always analyzed together with the plasma or blood sample of that same volunteer obtained after the placebo period. Therefore, differences between each supplement and the placebo were not affected by analytic variation between series.

Statistical analyses

For each subject, we calculated the differences between values for each of the 3 supplement periods and values for the placebo period. Statistical significance and 95% CIs of the mean differences were calculated by using Student's *t* test. Results were analyzed with use of SAS (version 6.12; SAS Institute, Inc, Cary, NC).

RESULTS

Ingestion of chlorogenic acid raised total homocysteine concentrations by 12% (1.2 μmol/L) in postprandial plasma and by 4% (0.4 μmol/L) in fasting plasma relative to placebo (Table 1, **Figure 1**). The rise of total homocysteine in postprandial plasma was 0.8-μmol/L (95% CI: 0.4, 1.2) higher than the rise in fasting plasma. Chlorogenic acid lowered the concentration of folate in fasting plasma by 8% (1.3 nmol/L; 95% CI: 0.6, 2.1) relative to placebo (**Table 2**). Concentrations of vitamins B-6 and B-12 were not significantly affected by chlorogenic acid.

Ingestion of black tea raised total homocysteine concentrations by 11% (1.1 μmol/L) in postprandial plasma and by 5% (0.5 μmol/L) in fasting plasma relative to placebo (Table 1, **Figure 2**). The rise in postprandial plasma was 0.6-μmol/L (95% CI: −0.0, 1.2) higher

TABLE 2
Concentrations of folate and vitamin B-12 in postprandial and fasting plasma and concentrations of vitamin B-6 in fasting whole blood collected from 20 healthy volunteers after ingestion of placebo, chlorogenic acid, black tea, or quercetin-3-rutinoside for 7 d each[1]

Supplement	Folate		Vitamin B-12		Vitamin B-6: fasting whole blood
	Fasting plasma	Postprandial plasma	Fasting plasma	Postprandial plasma	
	nmol/L		*pmol/L*		*nmol/L*
Placebo	22.6 ± 8.8	19.2 ± 5.0	235 ± 65	229 ± 66	81 ± 17
Chlorogenic acid	15.3 ± 4.8[2,3]	19.6 ± 5.8	247 ± 67[2]	224 ± 57	81 ± 14
Black tea	21.4 ± 6.9	19.5 ± 5.3	238 ± 65	239 ± 69	—[4]
Quercetin-3-rutinoside	20.0 ± 5.6[3]	19.3 ± 5.8	245 ± 82	233 ± 61	—[4]

[1] $\bar{x}$ ± SD. Postprandial blood samples were collected 4–5 h after the last supplement intake and fasting blood samples were collected ≈20 h after the last supplement intake.
[2] Folate and vitamin B-12 after chlorogenic acid were also measured in another series of analyses. The concentration of folate in plasma after placebo in this series of analyses was 16.7 ± 5.1 nmol/L and that of vitamin B-12 was 242 ± 67 pmol/L.
[3] Significantly different from placebo, $P < 0.05$.
[4] Not measured.

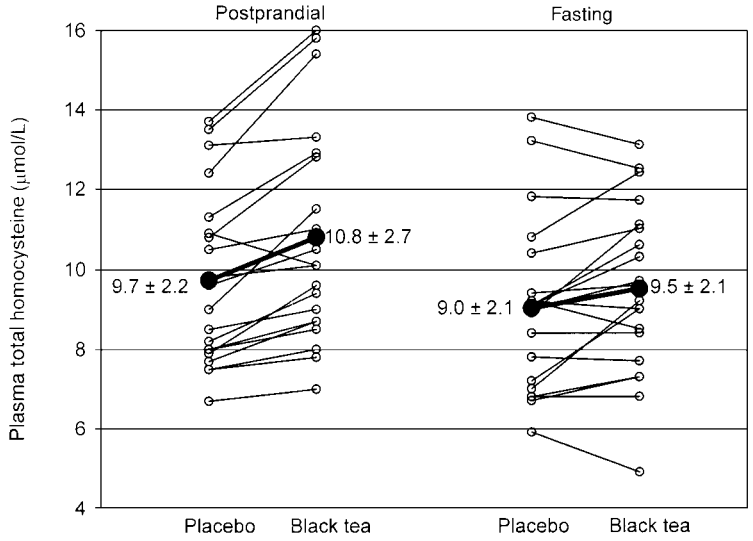

FIGURE 2. Plasma total homocysteine concentrations in 20 healthy subjects after they had consumed 4 g black tea solids (containing ≈4.3 mmol polyphenols) or a placebo daily, for 7 d each, in random order. Postprandial blood samples were collected 4–5 h after the last supplement intake and fasting samples were collected ≈20 h after the last supplement intake. Shown are the individual (○) and mean ± SD (●) changes.

than the rise in fasting plasma. Concentrations of B vitamins were not significantly affected by black tea (Table 2).

Ingestion of quercetin-3-rutinoside did not significantly affect total homocysteine concentrations in postprandial and fasting plasma (Table 1, **Figure 3**), but lowered the concentration of folate by 11% (2.5 nmol/L; 95% CI: 0.1, 5.0) in fasting plasma (Table 2). Concentrations of vitamins B-6 and B-12 were not significantly affected by quercetin-3-rutinoside. It is possible that we did not find an effect of quercetin-3-rutinoside on plasma homocysteine because the dose of quercetin-3-rutinoside used in this study was only 12–16% of the dose of chlorogenic acid and of black tea polyphenols. However, this dose of quercetin-3-rutinoside is ≈13 times higher than the average intake of quercetin in the population (16).

DISCUSSION

We found that consumption of 2 g chlorogenic acid/d by healthy humans raises homocysteine concentrations in postprandial plasma by 12% and in fasting plasma by 4%. Furthermore, we found that consumption of 4 g black tea solids/d also raises homocysteine concentrations in postprandial plasma by 11% and in fasting plasma by 5%. The dose of 2 g chlorogenic acid used in this study is comparable with ≈1.5 L strong coffee; the dose

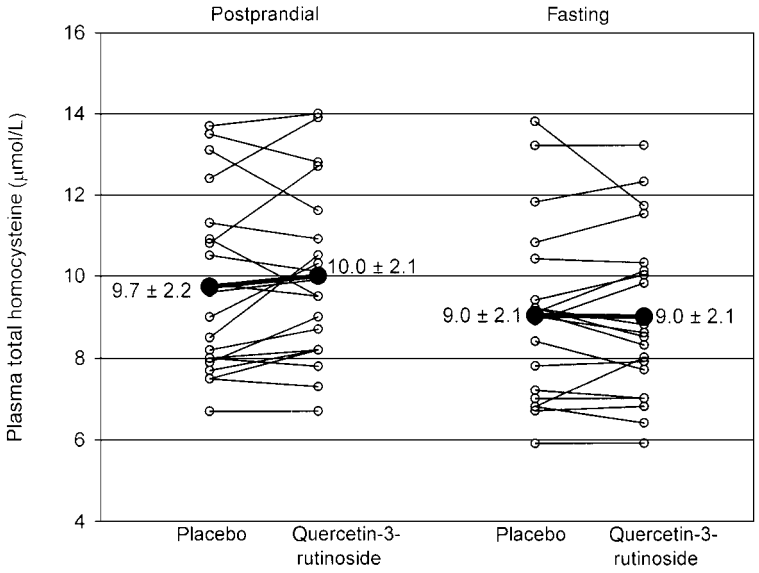

FIGURE 3. Plasma total homocysteine concentrations in 20 healthy subjects after they had consumed 440 mg (0.7 mmol) quercetin-3-rutinoside or a placebo daily, for 7 d each, in random order. Postprandial blood samples were collected 4–5 h after the last supplement intake and fasting samples were collected ≈20 h after the last supplement intake. Shown are the individual (○) and mean ± SD (●) changes.

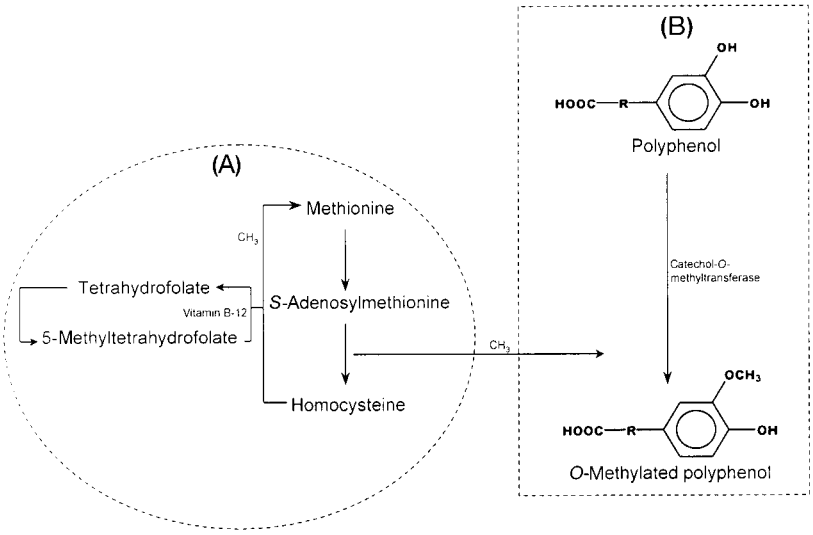

FIGURE 4. Proposed interaction between the hepatic metabolism of homocysteine (A) and the *O*-methylation of polyphenols (B).

of 4 g black tea solids is comparable with 2 L strong black tea. This implies that both black tea and coffee increase plasma homocysteine to the same extent. Thus, chlorogenic acid in coffee is at least partly responsible for the higher plasma homocysteine concentrations observed in coffee drinkers (3–7).

Chlorogenic acid and plasma total homocysteine

The rise in homocysteine in postprandial plasma of 12% after daily consumption of 2 g chlorogenic acid in this study was lower than the rise of 10–20% in 2 intervention studies after daily consumption of 1 L coffee, which contains ≈1 g chlorogenic acid (6, 7). The rise in plasma homocysteine concentrations in the present study was also lower than that found in epidemiologic studies (3–5). The fact that the rise in plasma homocysteine was lower in our study than in the studies with coffee might indicate that chlorogenic acid is not the only homocysteine-raising factor in coffee.

Beforehand, we did not know how long we had to give the supplements to induce an effect on plasma homocysteine. We expected that a supplementation period of 7 d would be long enough to stabilize plasma homocysteine because the half-life of elimination of plasma homocysteine after a methionine load is ≈12 h (25). In the present study we indeed showed that a supplementation period of 7 d is long enough to induce an effect on plasma homocysteine. However, we do not know the magnitude of the effect of chlorogenic acid on plasma homocysteine when chlorogenic acid is supplied for >7 d. Furthermore, we found that the effect of chlorogenic acid on homocysteine seems to subside within hours because the rise in homocysteine concentration in postprandial plasma samples, collected ≈5 h after ingestion of chlorogenic acid, was significantly higher than that in fasting plasma samples, collected ≈20 h after ingestion. We do not know whether the rise in homocysteine might have been even higher at other time points after intake because we collected blood only twice after ingestion of each supplement. We also saw a larger rise of homocysteine in postprandial plasma than in fasting plasma after the intake of black tea, although the fasting blood sample was collected ≈12 h after the intake of the last dose (1 g) of the black tea solids. It is possible that the rise in homocysteine was higher in the postprandial blood samples than in the fasting blood samples because the effect of chlorogenic acid and black tea on homocysteine metabolism is fast and short term, like the effect of methionine on plasma homocysteine (17). Other studies are necessary to establish the kinetics of the effect of chlorogenic acid and black tea on plasma homocysteine. We conclude that changes in homocysteine induced by chlorogenic acid or black tea apparently occur within hours rather than days and that the supplementation period of 7 d was adequate.

Black tea and plasma total homocysteine

We found that intake of 4 g black tea solids/d, containing 4.3 mmol polyphenols, raises homocysteine in postprandial plasma by 11% and in fasting plasma by 5%. The magnitude of these rises is similar to that observed after intake of 5.5 mmol chlorogenic acid. However, the dose of black tea used is higher than the mean daily consumption of black tea in the general population (26) and we do not know the effects of lower doses on plasma homocysteine.

We studied black tea because it is the tea most commonly consumed worldwide and because the only epidemiologic study that investigated the association between black tea consumption and homocysteine concentration indicated that black tea might affect plasma homocysteine (3). In future studies of the effect of tea polyphenols on plasma homocysteine, it might be interesting to study the effect of green tea. Whereas black tea contains mainly polymerized catechins, the major polyphenols in green tea are monomeric catechins (11).

Quercetin-3-rutinoside and plasma total homocysteine

In contrast with chlorogenic acid and black tea, quercetin-3-rutinoside did not significantly affect plasma homocysteine concentrations. This difference might be explained by the relatively low dose of quercetin-3-rutinoside used, which was only 12–16% of the dose of chlorogenic acid and of polyphenols from black tea. Nevertheless, the dose of 0.7 mmol quercetin-3-rutinoside is

≈13 times higher than the average quercetin intake in the general population, which is ≈0.05 mmol (16). Therefore, we believe that quercetin intake in the general population does not have a substantial effect on homocysteine concentrations, although we cannot exclude the possibility that doses of quercetin higher than used in our study might raise homocysteine. Quercetin-3-rutinoside lowered plasma folate concentrations by 11%. We do not have an explanation for this finding.

Mechanism of the effect of polyphenols on plasma total homocysteine

The mechanisms by which chlorogenic acid and black tea raise plasma homocysteine are not clear. First, changes in plasma homocysteine might be mediated by vitamin B-6, vitamin B-12, and folate, which are involved in the homocysteine pathway. We found that of these vitamins only folate was affected by the supplements. A decrease in plasma folate can lead to an increase in plasma homocysteine as a result of a decrease in remethylation of homocysteine into methionine (**Figure 4**) (27). A decrease in plasma folate, which could explain an increase in plasma homocysteine, was found in fasting plasma only after supplementation with chlorogenic acid and not black tea. In contrast, plasma folate also decreased in fasting plasma after quercetin-3-rutinoside, without a concomitant rise in plasma homocysteine. Furthermore, plasma folate concentrations in the postprandial blood samples were not significantly affected by the supplements, whereas the largest increase in plasma homocysteine was found in postprandial plasma. Thus, a direct role for folate in the homocysteine-raising effect of chlorogenic acid and black tea polyphenols is unlikely.

We speculate that *O*-methylation reactions that occur in the metabolism of polyphenols are involved in the homocysteine-raising effect of polyphenols. Such methylation reactions transfer a methyl group from *S*-adenosylmethionine to polyphenols and thereby produce homocysteine (Figure 4) (28). Thus, consumption of a high dose of polyphenols might increase homocysteine production through increased methylation reactions (29). This notion is supported by data from studies on L-dopa (L-3,4-dihydroxyphenylalanine). Like polyphenols, L-dopa is *O*-methylated. Indeed, both rats fed L-dopa and Parkinson disease patients treated with L-dopa have higher homocysteine concentrations than do control subjects (30–34). Thus, an increase in the *O*-methylation reactions in the body could result in higher plasma homocysteine concentrations, which might explain why high intakes of polyphenols raise plasma homocysteine.

Conclusion

High intakes of chlorogenic acid, which is present in coffee, and of black tea raise plasma homocysteine concentrations. Thus, chlorogenic acid in coffee might be at least partly responsible for the higher plasma homocysteine concentrations of coffee drinkers. A high plasma homocysteine concentration is a predictor of risk of cardiovascular disease (1, 2, 18). However, it is still unclear whether a high homocysteine concentration is causally related to cardiovascular disease or is merely an indicator of another process that causes cardiovascular disease.

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