Contribution of caffeine to the homocysteine-raising effect of coffee: a randomized controlled trial in humans

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ABSTRACT

Background: A high plasma total homocysteine concentration is associated with increased risk of cardiovascular disease. Consumption of unfiltered or filtered coffee raises total homocysteine concentrations in healthy volunteers. The responsible compound, however, is unknown.

Objective: The objective was to determine whether caffeine explains the homocysteine-raising effect of coffee.

Design: Forty-eight subjects aged 19–65 y completed this randomized crossover study with 3 treatments, each lasting 2 wk. Subjects consumed 6 capsules providing 870 mg caffeine/d (test treatment), 0.9 L paper-filtered coffee providing ~870 mg caffeine/d, or 6 placebo capsules. Blood samples were drawn fasting and 4 h after consumption of 0.45 L coffee or 3 capsules.

Results: The mean fasting plasma homocysteine concentration after the placebo treatment was 9.6 ± 3.1 μmol/L. The caffeine and coffee treatments increased fasting homocysteine by 0.4 μmol/L (95% CI: 0.1, 0.7; P = 0.04), or 5%, and by 0.9 μmol/L (95% CI: 0.6, 1.2; P = 0.0001), or 11%, respectively, compared with placebo. The increase in homocysteine concentrations 4 h after consumption of 0.45 L coffee relative to consumption of 3 placebo capsules was 19% (P = 0.0001). Caffeine treatment had a much weaker acute effect on homocysteine (4%; P = 0.09). Effects of caffeine were stronger in women than in men, but the effects of coffee did not differ significantly between men and women.


KEY WORDS Caffeine, paper-filtered coffee, homocysteine, B vitamins, crossover experiment

INTRODUCTION

Elevated plasma concentrations of total homocysteine predict an increased risk of cardiovascular disease (1, 2). However, it remains uncertain whether this relation is causal (3). Randomized clinical trials are underway to study whether a reduction in homocysteine concentrations with B vitamins reduces the risk of cardiovascular disease (4).

If elevated plasma concentrations of total homocysteine cause cardiovascular disease, it would be important to prevent elevated homocysteine concentrations in the general population. In several cross-sectional surveys, a positive association between heavy coffee drinking and plasma concentrations of total homocysteine was reported (5–7). For example, of 16 000 Norwegians, those who drank ≥9 cups coffee/d had plasma total homocysteine concentrations that were >20% higher than those who refrained from drinking coffee (5). An experiment with unfiltered coffee similar to the boiled coffee that was once commonly consumed throughout Scandinavia confirmed this finding (8). Another experiment by our group showed that regular paper-filtered coffee raised homocysteine concentrations to a similar extent and suggested that the effect occurred within a few hours after coffee consumption (9). Because a paper filter retains cafestol and kahweol, which are known to raise serum concentrations of cholesterol, it is unlikely that these coffee compounds are responsible for raising homocysteine concentrations. Caffeine, which passes through a paper filter, might be responsible. A recent observational study showed a positive association between caffeine intake and homocysteine concentrations (10).

In the present study we tested whether caffeine is responsible for the higher plasma concentrations of total homocysteine during consumption of paper-filtered coffee. Homocysteine was measured in the morning, after an overnight fast, and several hours after the consumption of caffeine capsules or coffee to investigate the time course of the effects.

SUBJECTS AND METHODS

Subjects and design

The study was conducted according to good clinical practice guidelines at TNO Nutrition and Food Research (Zeist, Netherlands). The protocol was approved by the local medical ethics committee.

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The subjects were recruited from a pool of volunteers registered at the Institute, and all gave written, informed consent. Subjects were eligible if they usually drank >6 cups regular filtered or instant (soluble) coffee daily; were between 18 and 65 y of age; had a body mass index (in kg/m\(^2\)) <30; consumed <28 alcohol-containing beverages per week if male and <21 if female; had no history of cardiovascular or gastrointestinal disease; were healthy on the basis of a physical examination, blood tests, and dipstick urinalysis; were not consuming a prescribed diet; had not used vitamin B supplements within 3 mo before entering the study; and had a plasma total homocysteine concentration <25 \(\mu\)mol/L.

We used a Latin-square design with 3 treatments given in random order for 2 wk each: caffeine (test treatment), paper-filtered coffee (positive control treatment), and placebo (negative control treatment). For caffeine and placebo treatment this was a double-blind study. Fifty-four volunteers were stratified by sex and fast- ing homocysteine concentration (measured at a screening visit, \(\approx1\) mo before the study) and were then randomly assigned to 1 of 6 treatment sequences.

Caffeine treatment consisted of 6 capsules (each containing 145 mg caffeine) providing a total of 870 mg caffeine/d. Coffee consumption was not allowed. Coffee could, by choice, be replaced by milk, herbal tea, or broth, for a maximum of 3 of these beverages per day in addition to the habitual amount used or water. Coffee treatment consisted of 0.9 L coffee/d, brewed with 1 L water and 52 g ground coffee. While designing our study, we prepared coffee in this way and the total amount of caffeine provided by this brew was measured to be \(\approx870\) mg. The ratio of 52 g ground coffee to 1 L water gave a less strong brew of coffee than did a ratio of 70 g ground coffee to 1 L water, which we used in our previous study (9). In that study, the coffee brew was considered too strong by several participants. In the present study, subjects were allowed to dilute the brew if they considered the coffee to be too strong. Three of the subjects reported diluting the coffee with hot water because it was too strong. Placebo treatment consisted of daily consumption of 6 capsules, each containing 145 mg cellulose (Avicel; Fagron, Nieuwerkerk aan den Yssel, Netherlands) coffee—a blend of arabica and robusta beans widely used in the Netherlands. The capsules were provided in 4 medication-dispenser boxes, each of which contained enough capsules for 4 d. The boxes contained 28 small compartments (ie, 7 compartments for each day, of which 6 contained capsules to be consumed; the seventh compartment contained a spare capsule). In addition to each treatment, the subjects were provided with packages of herbal tea (various types of Zonnatura brand, Zoetermeer, Netherlands), which contained herbs and spices such as mint, fennel seed, aniseed, and licorice. At the last visit of each treatment period, the remaining portions of coffee and capsules were returned and counted.

### Blood sampling and assays

Both fasting and nonfasting venous blood samples were collected on days 11 and 14 of each treatment period. After blood collection between 0800 and 0930 (ie, after the subjects had been fasting for \(\geq12\) h), the subjects consumed a standard breakfast consisting of 2 slices of whole-meal bread with low-fat margarine (one with jam and one with colored sugar sprinkles), a glass of orange juice, and one-sixth of the daily treatment (ie, 1 cup coffee or 1 capsule taken with 1 cup water). The subjects were then free to leave the institute but had to return 4 h later for a second blood collection (between 1200 and 1330). In the meantime, the subjects had to consume a snack (ie, a shortbread biscuit) that we provided and another 2 cups coffee (ie, they finished the 0.45 L coffee that had been prepared) or 2 cups water with 2 capsules. The subjects were not allowed to eat or drink anything else except tap water.

The fasting blood samples were used for homocysteine and B vitamin analyses, and the nonfasting blood samples were used for homocysteine and caffeine analyses. Caffeine concentrations were used to check compliance.

For plasma analyses, blood was collected in tubes containing EDTA. For serum analyses, blood was collected in tubes containing clot activator and a gel to separate serum and packed cells after centrifugation. Immediately after collection, the blood was mixed well and put on ice. Within 30 min of collection, all samples were centrifuged for 10 min at 2000 \(\times \) g at 4°C. Aliquots were stored at \(-15^\circ\)C. The samples were coded to hide the identity and treatment of the subjects from the laboratory technicians. All samples obtained from one subject were analyzed within the same run without compromising the blinding of the samples.

Total homocysteine was measured by HPLC (11, 12). Within- and between-run CVs were 3.6% and 6.4%, respectively. Vitamin B-6 was measured by HPLC (13), and folate and vitamin B-12 were measured with the SimulTRAC Radioassay Kit (ICN Pharmaceuticals, Orangeburg, NY). Intra- and interassay CVs were <8% for all vitamins. Caffeine in serum was measured by HPLC (ClinRep Komplettkit für Theophyllin, Theobromin und Coffein; Recipe Chemical + Instruments Labortechnik, Munich, Germany). Within- and between-run CVs were 2% and 7%, respectively.

### Statistics

Treatment effects were studied first with analysis of variance (ANOVA). If the ANOVA indicated an overall treatment effect (\(P \leq 0.05\)), the treatment means were compared with one-sided, paired \(t\) tests. For each subject, the values obtained on days 11 and 14 of each treatment were averaged. The responses were calculated by subtracting the subject’s average value at the end of the placebo treatment from that at the end of the caffeine or coffee treatment.

The aim of our study was to test the hypothesis that caffeine is responsible for the homocysteine-raising effect of coffee, ie, 1)
coffee raises homocysteine, 2) caffeine raises homocysteine, and 3) the effect of caffeine is equal to that of coffee. Statement 1 is a logical consequence of statements 2 and 3; therefore, statement 1 is redundant. Hence, statement 1 was dropped and we were left with 2 comparisons for which a Bonferroni correction should be made, ie, dividing the significance level α by 2. We tested one-sided because our hypothesis was that caffeine would either raise homocysteine or fail to affect it; we considered it unlikely, a priori, that caffeine would lower homocysteine. We therefore used α = 0.05 (ie, 0.10 divided by 2). Setting the α level to < 0.05 could introduce a type 2 error, ie, a false-negative conclusion. Carryover effects were tested for and found to be absent. Subject characteristics and serum concentrations of caffeine are presented descriptively. SAS software (version 6.12; SAS Institute Inc, Cary, NC) was used.

RESULTS

Subject characteristics

Forty-eight subjects (21 men, 27 women) completed the trial; 15 (31%) were smokers. The other baseline characteristics, including fasting plasma concentrations of total homocysteine, are shown in Table 1. The 6 groups of different treatment order had similar baseline concentrations of fasting homocysteine, indicating that randomization was successful.

Serum caffeine concentrations

The mean (± SD) concentrations of caffeine in the nonfasting serum samples obtained at the end of each treatment period were 64.4 ± 23.2 µmol/L after caffeine, 54.1 ± 21.1 µmol/L after coffee, and 1.0 ± 3.6 µmol/L after placebo. Caffeine was detected in the serum samples of 5 subjects at concentrations of 1.6, 2.6, 11.3, 15.5, and 16.5 µmol/L during the placebo period. The subject with the caffeine concentration of 11.3 µmol/L admitted to having consumed 2 chocolates, but the other subjects reported no deviations from the dietary restrictions. Because the serum caffeine concentrations of these 5 subjects were still much lower than the mean concentrations during the caffeine and coffee treatments, their values were not excluded. We concluded that adherence to the protocol was generally satisfactory.

Metabolism of caffeine in smokers is known to be more rapid than that in nonsmokers. Indeed, we found that in women not using oral contraceptives, nonfasting caffeine concentrations were 17–35% lower in smokers than in nonsmokers. As expected, we also observed higher caffeine concentrations in users than in nonusers of oral contraceptives.

Plasma homocysteine and vitamin concentrations

Mean (± SD) fasting and nonfasting plasma concentrations of total homocysteine during each of the 3 treatment periods are shown in Table 2, as are the mean differences in homocysteine concentrations between the caffeine or coffee treatment periods and the placebo treatment period. These differences are expressed as percentages relative to the placebo period in Figure 1.

A two-factor ANOVA with treatment and fasting status (yes or no) as factors showed an interaction between treatment and fasting status (P < 0.0001), as is indicated by the results described below. Caffeine treatment increased fasting homocysteine concentrations by 0.4 ± 1.0 µmol/L (95% CI: 0.1, 0.7 µmol/L; P = 0.04), or 5%. The effect of coffee was about twice as strong as that of caffeine: the mean fasting plasma concentration of total homocysteine increased by 0.9 ± 1.2 µmol/L (95% CI: 0.6, 1.2 µmol/L; P = 0.0001), or 11%. The effect of caffeine on fasting homocysteine was significantly less than the effect of coffee (P < 0.001).

As can be observed in Table 2, mean homocysteine concentrations were lower in the nonfasting state than in the fasting state during the placebo and caffeine treatments, but not during the coffee treatment. Thus, coffee drinking, and to a lesser extent caffeine treatment, appeared to interfere with the decrease in homocysteine concentrations after breakfast and snacks. Caffeine tended to increase nonfasting homocysteine by 0.3 ± 1.2 µmol/L (95% CI: −0.1, 0.6 µmol/L; P = 0.09), or 4%; a similar effect on fasting homocysteine concentrations was seen. In contrast with this effect, coffee drinking had a stronger effect on nonfasting plasma concentrations of total homocysteine than on fasting concentrations; the increase was 1.5 ± 0.9 µmol/L (95% CI: 1.3, 1.8 µmol/L; P = 0.0001), or 19%, compared with placebo. The effect of caffeine on nonfasting homocysteine was significantly less than the effect of coffee (P < 0.0001).

We tested whether men and women differed in their response to caffeine or coffee, although this was not based on an a priori hypothesis. The effect of caffeine treatment was mainly limited to women: they showed an increase in fasting homocysteine concentrations of 0.6 ± 0.9 µmol/L (95% CI: 0.3, 1.0 µmol/L), or 8%, whereas men showed an increase of 0.0 ± 1.1 µmol/L (95% CI: −0.4, 0.4 µmol/L), or 1%. For nonfasting homocysteine, the responses were 0.6 ± 1.3 µmol/L (95% CI: 0.3, 0.9 µmol/L), or 7%, in women and −0.1 ± 0.9 µmol/L (95% CI: −0.5, 0.3 µmol/L), or 0%, in men. This interaction between caffeine treatment and sex was marginally significant (P = 0.06 for fasting homocysteine and P = 0.10 for nonfasting homocysteine).

Women had higher mean serum caffeine concentrations during both caffeine and coffee treatments than did men: 72.1 ± 25.2 compared with 54.1 ± 16.0 µmol/L (P < 0.005) with caffeine and 61.8 ± 23.7 compared with 43.8 ± 11.8 µmol/L (P < 0.005) with coffee. The homocysteine response to coffee treatment was not significantly different between men and women (data not shown). The circulating concentrations of vitamin B-6, vitamin B-12, and folate were not affected significantly by the coffee or caffeine treatment (Table 2).

DISCUSSION

We found that consumption of pure caffeine increased homocysteine concentrations in healthy subjects, but caffeine had only 25–50% of the homocysteine-raising effect of paper-filtered coffee with a similar amount of caffeine. Coffee, unlike caffeine, affected homocysteine concentrations within hours after intake. Thus, compounds in coffee other than caffeine must be additionally responsible for the homocysteine-raising effect of coffee. With respect to coffee, this study confirmed the findings of our previous study—a randomized, controlled, crossover study of 26
TABLE 2
Blood concentrations of total homocysteine, in the fasting state and shortly after treatment, and of vitamins in healthy subjects who consumed placebo, caffeine capsules, or 0.9 L paper-filtered coffee for 2 wk.

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Caffeine</th>
<th>Coffee</th>
<th>( P ) (ANOVA) ( ^2 )</th>
<th>Caffeine minus placebo</th>
<th>Coffee minus placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma total homocysteine (μmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>9.6 ± 3.1</td>
<td>10.0 ± 3.1</td>
<td>10.5 ± 3.1</td>
<td>&lt;0.0001</td>
<td>0.4 ± 1.0 (0.1, 0.7)</td>
<td>0.9 ± 1.2 (0.6, 1.2)</td>
</tr>
<tr>
<td>Nonfasting</td>
<td>8.9 ± 2.9</td>
<td>9.2 ± 3.0</td>
<td>10.5 ± 3.1</td>
<td>&lt;0.0001</td>
<td>0.3 ± 1.2 (0, 1.6)</td>
<td>1.5 ± 0.9 (1.3, 1.8)</td>
</tr>
<tr>
<td>Serum folate (nmol/L)</td>
<td>13.3 ± 3.7</td>
<td>13.7 ± 4.1</td>
<td>13.3 ± 4.4</td>
<td>0.23</td>
<td>0.4 (0, 1.0)</td>
<td>0.1 (0, 0.6)</td>
</tr>
<tr>
<td>Plasma vitamin B-6 (nmol/L)</td>
<td>57 ± 29</td>
<td>60 ± 46</td>
<td>55 ± 33</td>
<td>0.54</td>
<td>3 (5, 10)</td>
<td>-2 (9, 6)</td>
</tr>
<tr>
<td>Serum vitamin B-12 (pmol/L)</td>
<td>308 ± 92</td>
<td>315 ± 89</td>
<td>309 ± 88</td>
<td>0.35</td>
<td>7 (3, 17)</td>
<td>1 (9, 11)</td>
</tr>
</tbody>
</table>

\(^1\) ± SD; 95% CI in parentheses. \( n = 48 \). Each subject received each treatment in a multiple crossover design.

\(^2\) A two-factor ANOVA with treatment and fasting status (yes or no) as factors showed an interaction between treatment and fasting status (\( P < 0.0001 \)), which justified a separate analysis of the effects on fasting and nonfasting homocysteine concentrations. Treatment effects were studied first with ANOVA. If the ANOVA indicated an overall treatment effect (\( P \leq 0.05 \)), treatment means were compared with Bonferroni-corrected, one-sided paired \( t \) tests.

healthy subjects—in which we observed an increase in mean fasting homocysteine of 18% after consumption of paper-filtered coffee for 3–4 wk (9). In our previous study, coffee increased nonfasting homocysteine concentrations by 30% (R Urgert, T Van Vliet, PL Zock, MB Katan, unpublished observations, 1999). However, in that study, we used 70 g ground coffee instead of 52 g, as in the present study. Together with the shorter intervention period, the lower strength of the coffee might explain the weaker effect observed in the present study. In both studies, the changes in fasting homocysteine concentrations were not accompanied by changes in fasting circulating vitamin concentrations.

For proper interpretation of the present findings, it is crucial that the study subjects were compliant and that the caffeine doses were similar during the coffee and caffeine-capsule treatments. During the placebo treatment, no caffeine was detected in the nonfasting serum samples of most of the subjects (43 of 48), whereas caffeine was present in serum in substantial amounts during treatment with caffeine or coffee. Thus, the study participants appeared to have adhered to the protocol in a satisfactory manner. Serum caffeine concentrations increased by \( \approx 15–20\% \) more after consumption of the caffeine capsules than after coffee drinking. This finding may have been due to differences in the absorption of caffeine from the coffee and caffeine capsules. However, we believe that the consumption of and serum concentrations of caffeine during the coffee and coffee treatment periods were similar enough to make a valid comparison of their effects on homocysteine.

We had no a priori hypothesis that the effect of caffeine on homocysteine concentrations would differ between men and women. However, we observed that the effect of caffeine was virtually limited to women. This finding agrees with the observation that women had somewhat higher serum concentrations of caffeine than men. Nevertheless, the distribution of serum caffeine concentrations in men and women largely overlapped, so it is unlikely that internal caffeine doses reached a critical concentration in women only. We have no clear explanation for this observation; it may have been due to chance.

To obtain information about the time course of the effect of coffee drinking (or caffeine) on homocysteine concentrations, we collected blood samples before (fasting) and several hours after consumption of coffee or caffeine capsules (nonfasting). Both during the placebo and caffeine periods, the mean plasma concentrations of homocysteine were lower during the nonfasting than during the fasting state. Homocysteine lowering in response to a breakfast was observed in other studies (14, 15). In our study, coffee drinking appeared to interfere with this normal decrease in homocysteine concentrations after a breakfast.

What mechanism could explain the homocysteine-raising properties of caffeine? Caffeine is a methylxanthine, and it might act as a vitamin B-6 antagonist, as does theophylline (16). In this way, it could compromise the breakdown of homocysteine through the vitamin B-6-dependent transsulfuration pathway. We observed no change in the concentration of vitamin B-6 after caffeine consumption; however, this antagonistic effect of caffeine is probably not reflected by changes in plasma vitamin B-6.

Caffeine (and thus coffee) is a diuretic. However, the diuretic effect of caffeine does not explain the acute increase in homocysteine concentrations observed after coffee consumption, because no increase in homocysteine was seen after an equal intake of caffeine from capsules (Table 2).

If caffeine is responsible for, at the most, one-half of the homocysteine-raising effect of coffee, what other factors are likely candidates?
We had already ruled out the diterpenes cafestol and kahweol, which are known for their cholesterol-raising effects (17), because they are retained by a paper filter. Another compound that is predominantly derived from coffee is chlorogenic acid, a polyphenolic compound (18). We previously reported (19) that chlorogenic acid raises homocysteine: consumption of 2 g chlorogenic acid/d for 7 d—an amount equivalent to ~1.5 L strong coffee/d—increased fasting homocysteine concentrations by 4% and nonfasting homocysteine concentrations (after a hot meal) by 12%. We suggested that O-methylation reactions that occur during the metabolism of chlorogenic acid are responsible for the homocysteine-raising effect. In that study, the effects were more apparent in the nonfasting than in the fasting state, just as we observed with coffee consumption. Taken together, chlorogenic acid and caffeine together probably account for most of the effect of coffee on homocysteine.

In conclusion, caffeine is partly responsible for the homocysteine-raising effect of coffee. The effect of coffee appears to be more pronounced several hours after coffee consumption than after an overnight fast. Epidemiologic associations between coffee consumption and cardiovascular disease are conflicting (20, 21). Thus, the public health implications of the homocysteine-raising effect of coffee. The effect of coffee appears to be more apparent in the nonfasting than in the fasting state, just as we observed with coffee consumption. Taken together, chlorogenic acid and caffeine together probably account for most of the effect of coffee on homocysteine.

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