Heavy coffee consumption and plasma homocysteine: a randomized controlled trial in healthy volunteers

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ABSTRACT

Background: An elevated plasma concentration of total homocysteine is considered to be a strong risk factor for cardiovascular disease. Heavy coffee drinking has been related to high homocysteine concentrations in epidemiologic studies and in one experiment in which healthy subjects drank unfiltered, boiled coffee.

Objective: Our goal was to determine whether daily consumption of paper-filtered coffee raises plasma concentrations of total homocysteine in healthy subjects.

Design: Twenty-six volunteers (18–53 y of age) consumed 1 L/d of paper-filtered coffee brewed with 70 g regular ground beans or no coffee for 4 wk each in a randomized, crossover design.

Results: The mean ± SD plasma concentration of total homocysteine in fasting blood was 8.1 ± 1.8 μmol/L after abstention from coffee and 9.6 ± 2.9 μmol/L after 3–4 wk of coffee drinking, a difference of 1.5 μmol/L (95% CI: 0.9, 2.1 μmol/L) or 18% (P < 0.001). Coffee increased homocysteine concentrations in 24 of 26 individuals. Circulating concentrations of vitamin B-6, vitamin B-12, and folate were unaffected.

Conclusion: Drinking large quantities of paper-filtered coffee raises fasting plasma concentrations of total homocysteine in healthy individuals.

KEY WORDS Coffee consumption, homocysteine metabolism, dietary factors, crossover experiment, risk factors

INTRODUCTION

A high plasma concentration of total homocysteine (the sum of all forms of homocysteine present in blood plasma) is considered to be a strong and independent risk factor for coronary, cerebral, and peripheral vascular disease (1–3). Epidemiologic studies indicate that a 10% increase in plasma concentration may be associated with a 10–15% increase in disease risk (2). The cause of an elevated concentration may be genetic, such as a mutation in genes encoding for homocysteine-metabolizing enzymes, or nongenetic, such as deficiencies in vitamin B-12, vitamin B-6, and folate (3).

The results of recent observational surveys suggest that a link may exist between coffee drinking habits and plasma homocysteine (4–6). Among 16 000 Norwegians, plasma concentrations of total homocysteine showed a dose-dependent relation with coffee intake, and subjects drinking ≥9 cups of coffee daily had >20% higher total homocysteine concentrations than did those who drank no coffee (4). An experiment with unfiltered coffee similar to the boiled coffee that was once commonly consumed throughout Scandinavia, and still is consumed in particular regions, showed that heavy intake of such coffee may indeed raise plasma total homocysteine (7). However, intake of such coffee is rare; even in Scandinavia, most people nowadays drink paper-filtered coffee (4). We therefore tested whether a high intake of paper-filtered coffee raises the plasma concentration of total homocysteine in healthy subjects.

SUBJECTS AND METHODS

Subjects and design

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

Subjects were recruited from a pool of volunteers registered at the institute and all gave their written, informed consent. Subjects were eligible if they usually drank between 5 and 8 cups of regular filtered or instant (soluble) coffee daily; were between 18 and 60 y of age; had a body mass index (in kg/m²) <32; consumed <28 alcohol-containing beverages per week for males and 21 for females; had no history of cardiovascular or gastrointestinal disease; were healthy as assessed by a physical examination, blood tests, and dipstick urinalysis; were not consuming a prescribed diet; had not used vitamin B supplements within 3 mo of entering the study; and had a plasma concentration of total homocysteine <20 μmol/L.

Forty volunteers met our criteria. They were stratified by sex and homocysteine concentration and then randomly assigned to

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One group consumed 1 L paper-filtered coffee/d for 4 wk (coffee period) and abstained from coffee during the next 4 wk (no-coffee period). The other group received the same treatments in reverse order. At the start of the second period, the amount of coffee was gradually increased from none to 1 L/d over the first 3 d in subjects switched to coffee treatment and similarly diminished in those subjects switched to no coffee. During the no-coffee period, subjects consumed a maximum of 3 cups/d each of milk, herbal tea, or broth, and water. Caffeine-containing products (chocolate, chocolate drinks, cola, tea, and certain painkillers) were prohibited during the entire trial. Subjects were asked to not change their dietary habits during the trial.

Seven subjects withdrew during the coffee periods because of nausea, restlessness, or sleeping problems and the medical ethics committee excluded 3 subjects who may have been susceptible to adverse effects of continued caffeine intake. Three subjects withdrew from the study for reasons unrelated to treatment (2 had a fever and 1 had acute appendicitis) and 1 subject was no longer available. Thus, 26 subjects completed the trial. Exclusions and dropouts occurred before homocysteine or other outcome measures had been analyzed.

Coffee preparation

Subjects were given an electric drip-filter coffee maker (Tomado Compact 500; Tomado, Zwijndrecht, Netherlands), paper filters, a 0.5-L insulating container, and household scales. Each week, subjects received a package of 500 g finely ground coffee. We used Douwe Egberts Roodmerk brand (Sara Lee/DE, Utrecht, Netherlands), a blend of arabica and robusta beans used widely in the Netherlands. Subjects prepared and consumed 1 L coffee brewed with 70 g grounds each day, which equals 6 large mugs of strong coffee. Coffee packages were returned to the institute at each weekly visit and were then weighed. For 3 subjects who had difficulty in complying, the dose was reduced from 70 to 60 g grounds/L brew.

Blood sampling and assays

Venous blood was collected from all subjects after they had fasted overnight on days 14, 21, 25, and 28 of both treatment periods. Nonfasting venous blood was taken at 1200 on day 7 of both periods. Heparin-treated blood was put on ice immediately after venipuncture. Plasma was separated within 30 min and aliquots were stored at −20°C until after the trial. Samples were coded so as to blind the laboratory technicians to the identity and treatment of the subjects, and all samples obtained from one subject were analyzed within the same run.

Total homocysteine concentrations were measured by HPLC (8, 9). Within- and between-run CVs were 3.5% and 8%, respectively. Pyridoxal-P (vitamin B-6) was also measured by HPLC (10) and folate and vitamin B-12 were measured with the Simul-TRAC Radioassay Kit (ICN Pharmaceuticals, Orangeburg, NY). Intra- and interassay CVs were <10% for all vitamins. Caffeine was measured by HPLC (ClinRep Komplettkit für Theophyllin, Theobromin und Coffein; Recipe Chemical + Instruments Labortechnik, Munich, Germany).

Statistics

For each subject, the plasma total homocysteine values obtained at the end of each period (days 21, 25, and 28) were averaged and the response calculated as the average value at the end of the coffee period minus that at the end of the no-coffee period. Carryover or period effects were tested for and found to be absent. Responses were therefore compared with zero by using paired t tests (SAS software version 6.12; SAS Institute Inc, Cary, NC).

RESULTS

Twenty-six subjects (10 men and 16 women) completed the trial. The subjects’ mean (±SD) age was 37 ± 12 y and their mean body mass index was 23 ± 3. Eleven subjects smoked.

During the coffee period, the mean caffeine concentration in nonfasting serum collected at noon on day 7 of treatment was 50.5 ± 32.4 μmol/L (range: 1.0–129.3 μmol/L); that in fasting serum collected on day 21 of treatment was 17.0 ± 16.5 μmol/L (range: 0.0–50.5 μmol/L). During the no-coffee period, minor traces of caffeine were detected in nonfasting serum of 3 individuals (0.5, 0.7, and 1.7 μmol/L, respectively) and in fasting serum of 1 individual (2.0 μmol/L). Adherence to the protocol thus appeared to have been satisfactory.

Heavy coffee drinking raised the fasting plasma concentration of total homocysteine in 24 of 26 subjects (Figure 1): the mean rose by 1.5 ± 1.5 μmol/L (95% CI: 0.9, 2.1 μmol/L) after 3–4 wk of coffee drinking (Table 1). Treatment order did not affect outcome: the mean increase was 1.8 ± 2.2 μmol/L in subjects who were switched from no coffee to coffee (n = 15) and 1.3 ± 0.8 μmol/L in those switched from coffee to no coffee (n = 11). Results were similar after only 2 wk: coffee drinking increased plasma total homocysteine by 22 ± 23% after 2 wk (range: −14% to 77%) and by 18 ± 16% after 3–4 wk (range: −2% to 67%). Circulating concentrations of B vitamins did not differ significantly between treatment periods.

DISCUSSION

We found that daily consumption of 1 L of strong, paper-filtered coffee increased the mean fasting plasma concentration
of total homocysteine by $\approx 20\%$ in healthy volunteers. The effect was seen within 2 wk.

It is unlikely that our study was confounded by changes in vitamin status because blood concentrations of folate, vitamin B-6, and vitamin B-12 did not differ significantly between treatment periods. The crossover design of our study should also have eliminated bias due to random drifts over time or to other dietary or environmental factors that might influence homocysteine concentrations. The number of dropouts suggests that our coffee regimen was too severe for some of our volunteers, even though we used this regimen without problems in previous trials of healthy volunteers recruited from the general population (7, 11). Theoretically, the 7 subjects who dropped out because of side effects of caffeine might have been exceptionally resistant to the homocysteine-raising effect of coffee. Although we find this unlikely, we cannot rule it out, and therefore our outcome might be valid only for subjects who tolerate high intakes of coffee.

Both unfiltered (7) and filtered coffee appear to contain a factor that raises plasma homocysteine. This factor is potentially also present in soluble (instant) coffee, espresso, and other types of coffee, but this will remain uncertain until the responsible compound is identified.

Few individuals drink coffee at the strength or in the amounts used here, amounting to $\approx 1100$ mg caffeine/L. Ten to 15 cups of regular strength coffee are needed to provide such amounts of caffeine (12, 13). Our findings are thus in line with those from an observational survey in which subjects used to drinking coffee were compared with those who abstained from coffee (4). The finding that chronic users of large amounts of coffee have higher homocysteine concentrations also suggests that the homocysteine-raising effect of coffee does not subside with chronic use.

A high homocysteine concentration predicts an increased risk of cardiovascular disease. However, prospective studies have not shown a convincing link between consumption of filtered coffee and risk of coronary artery disease (14). Consumption of unfiltered coffee does show an association with coronary artery disease (15), but that is explained by the cafestol that is present in unfiltered coffee and that raises cholesterol (11, 16). One explanation for the lack of an association between filtered coffee intake and coronary artery disease may be that the amount of coffee consumed in the studies that examined such an association was too low (14). However, it may also suggest that plasma homocysteine itself is innocuous and that elevated concentrations are merely a side effect of some other process that does cause cardiovascular disease. Randomized clinical trials are underway to answer the question of whether homocysteine is causal (17).

We conclude that drinking large quantities of coffee raises homocysteine in plasma. Whether this raises the risk of cardiovascular disease is not yet certain.

We thank the volunteers for their participation, all those involved at the TNO Nutrition and Food Research Institute for their dedication, and the laboratory staff at Wageningen University and Maastricht University for careful analyses.

### REFERENCES


### TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>No-coffee period</th>
<th>Coffee period</th>
<th>Change (95% CI)</th>
<th>P</th>
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<tbody>
<tr>
<td>Plasma total homocysteine ($\mu$mol/L)</td>
<td></td>
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<tr>
<td>After 2 wk</td>
<td>8.4 $\pm$ 2.3$^2$</td>
<td>10.0 $\pm$ 2.8</td>
<td>1.8 $\pm$ 1.6$^2$ (1.1, 2.4)</td>
<td>$&lt;0.001$</td>
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<tr>
<td>After 3–4 wk</td>
<td>8.1 $\pm$ 1.8</td>
<td>9.6 $\pm$ 2.9</td>
<td>1.5 $\pm$ 1.5 (0.9, 2.1)</td>
<td>$&lt;0.001$</td>
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<td>Serum folate (nmol/L)$^3$</td>
<td>14.6 $\pm$ 5.1</td>
<td>14.1 $\pm$ 5.2</td>
<td>$-0.5 \pm 2.8$ (–1.7, 0.6)</td>
<td>0.45</td>
</tr>
<tr>
<td>Plasma vitamin B-6 (nmol/L)$^3$</td>
<td>53 $\pm$ 36</td>
<td>49 $\pm$ 36</td>
<td>$-3 \pm 31$ (–16, 9)</td>
<td>0.56</td>
</tr>
<tr>
<td>Serum vitamin B-12 (pmol/L)$^3$</td>
<td>257 $\pm$ 92</td>
<td>251 $\pm$ 84</td>
<td>$-5 \pm 41$ (–22, 11)</td>
<td>0.58</td>
</tr>
</tbody>
</table>

$^1$x $\pm$ SD; $n = 26$.

$^2$Value missing for 1 subject.

$^3$Mean of values obtained after 14, 21, and 28 d.