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, or folate (3).

The results of recent observational surveys suggest that a link may exist between coffee drinking habits and plasma homocysteine (4–6). Among 16000 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...
Subjects were given an electric drip-filter coffee maker (TomoCompact 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, a package of 500 mL of milk, a 0.5-g packet of 35% sugar, a 0.5-g package of erythritol, a 0.5-g package of cocoa, a 0.5-g package of milk chocolate, a 0.5-g package of salt, a 0.5-g packet of chocolate drink mix, a 0.5-g packet of cola, a 0.5-g packet of tea, and a 0.5-g packet of painkillers. Caffeine-containing products (chocolate, coffee, 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 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 (1 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 measurements had been analyzed.

Coffee preparation

Subjects were given an electric drip-filter coffee maker (TomoCompact 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 SimulTRAC 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 was 10.4 ± 16.3 μmol/L (range: 0.0–129.3 μmol/L); that in fasting serum was 17.0 ± 16.5 μmol/L (range: 0.0–50.5 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 increase was 1.8 ± 2.2 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 in 26 healthy volunteers participating in a crossover study in which drinking 1 L of strong, paper-filtered coffee/d for 4 wk was compared with a 4-wk period of no coffee use.
Plasma vitamin B-6 (nmol/L)  
Serum folate (nmol/L)  
other process that does cause cardiovascular disease. Random-
ized 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

Fasting blood concentrations of total homocysteine and vitamins in healthy volunteers who consumed 1 L of strong, paper-filtered coffee or no coffee for 4 wk each in a crossover design

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

± SD; n = 26.

Values missing for 1 subject.

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