

# Unfiltered coffee increases plasma homocysteine concentrations in healthy volunteers: a randomized trial<sup>1-3</sup>

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## ABSTRACT

**Background:** An elevated plasma homocysteine concentration is a putative risk factor for cardiovascular disease. Observational studies have reported an association between coffee consumption and plasma homocysteine concentrations.

**Objective:** We studied the effect of coffee consumption on plasma homocysteine in a crossover trial. We used unfiltered coffee so as to include the possible effects of coffee diterpenes, which are removed by filtering.

**Design:** Sixty-four healthy volunteers (31 men and 33 women) with a mean ( $\pm$ SD) age of  $43 \pm 11$  y were randomly assigned to 2 groups. One group ( $n = 30$ ) drank 1 L unfiltered cafetière (French press) coffee daily for 2 wk. Such coffee is rich in the cholesterol-raising diterpenes kahweol and cafestol. The other group ( $n = 34$ ) received water, milk, broth, tea, and chocolate drinks instead of coffee. After a washout period of 8 wk, both groups received the alternate intervention for another 2 wk.

**Results:** Consumption of 1 L unfiltered coffee/d for 2 wk significantly raised fasting plasma homocysteine concentrations by 10%, from 12.8 to 14.0  $\mu\text{mol/L}$ .

**Conclusions:** Unfiltered coffee increases plasma homocysteine concentrations in volunteers with normal initial concentrations. It is unclear whether the effect is caused by the cholesterol-raising diterpenes present exclusively in unfiltered coffee or by factors that are also present in filtered coffee. *Am J Clin Nutr* 2000;71:480-4.

**KEY WORDS** Unfiltered coffee, homocysteine, diet, diterpenes, kahweol, cafestol, cardiovascular disease risk, humans, Netherlands

## INTRODUCTION

An elevated plasma homocysteine concentration is a putative risk factor for coronary, cerebral, and peripheral vascular disease (1, 2). Boushey et al (3) reported in a meta-analysis of homocysteine and cardiovascular disease that 10% of all coronary artery disease events may be explained by an elevated concentration of total plasma homocysteine. An elevated plasma homocysteine concentration can be caused by genetic defects—eg, a mutation in the methylenetetrahydrofolate reductase enzyme or heterozygosity for the cystathionine  $\beta$ -synthase deficiency—and by nongenetic factors. Examples of nongenetic factors are deficiencies in vitamin B-12, folate, and vitamin B-6 (4).

Other dietary factors might also affect plasma homocysteine. A positive correlation between the plasma homocysteine concentration and coffee consumption was reported in 2 other studies (5, 6). In Norway, 5916 healthy men and 6349 women aged 40–42 y were studied. Average plasma homocysteine was 9.1  $\mu\text{mol/L}$  in coffee abstainers and 11.2  $\mu\text{mol/L}$  in heavy coffee consumers, ie, those who drank >8 cups coffee/d (5). Filtered coffee was purportedly consumed by 96.4% of the subjects. No association was observed between decaffeinated coffee consumption and plasma homocysteine. This suggests that the effect on homocysteine is due to caffeinated coffee. In the United States, an older population of 151 women and 109 men (median age: 64 y) was investigated. The average plasma homocysteine concentration was 9.8  $\mu\text{mol/L}$  in coffee abstainers and 11.1  $\mu\text{mol/L}$  in a population drinking an average of 4 cups coffee/d (6). No detailed information on the type of coffee brew was reported in this study.

However, an association between coffee consumption and plasma homocysteine could not be confirmed in participants of the Atherosclerosis Risk in Communities Study in the United States (7). In this study, there was also no detailed information on the type of coffee brew reported. The inconsistencies between these observational studies suggest that not all types of coffee brew have the same effect on plasma homocysteine concentrations or that the effect is spurious. We therefore studied the effect of coffee on total plasma homocysteine in healthy volunteers in a placebo-controlled crossover trial. This study was part of a study in which we investigated the influence of coffee on biomarkers for colonic cancer. We used unfiltered coffee.

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fee so as to include all possible components, including diterpenes, which are known to raise cholesterol concentrations (8).

## SUBJECTS AND METHODS

### Subjects and design

The study protocol was approved by the local human ethics committee. We recruited healthy volunteers through an article in a local paper and posters on the hospital and faculty grounds. Subjects were eligible if they drank an average of >2–3 cups filtered coffee/d; were between 24 and 70 y of age; were not following a medical diet; used no laxatives, nonsteroidal antiinflammatory drugs, vitamin supplements, or lipid-reducing drugs; and had not used antibiotics within 2 mo of entering the study. Exclusion criteria were a history of liver or kidney diseases (serum alanine aminotransferase activity >30 U/L or a serum creatinine concentration above the upper limit of normal), a history of cholecystectomy or partial bowel resection, a serum total cholesterol concentration >6.5 mmol/L or fasting triacylglycerol concentrations >3.0 mmol/L, or living >50 km from the study site.

One hundred seventy-five volunteers applied for the study and filled out a medical questionnaire. Eighty-three subjects were not eligible because they did not meet the inclusion criteria. Ninety-two volunteers were investigated with a detailed medical history, a physical examination, and blood tests. Seventy volunteers were eligible and gave their written informed consent. Subjects were stratified by sex and smoking habit and then randomly assigned to the 2 treatment sequences. Subjects were enrolled in 3 shifts in 1997: one shift started in April, one in June, and one in September.

Subjects consumed their regular diets during the study but were instructed to maintain their usual diet. We checked compliance with a 3-d dietary recall in each intervention period. Diet composition was calculated by using the Dutch nutrient database (9).

The study consisted of 2 intervention periods of 2 wk each separated by a washout period of 8 wk in a crossover design. One group started by consuming 1 L unfiltered cafetière coffee/d, which is also known as French press or plunger coffee. (One liter of coffee equals 6 large cups.) The other group did not consume coffee, but instead consumed water, milk, chocolate drinks, tea, or broth; ≤3 cups each of these beverages was to be consumed daily. On day 15 of each intervention period, fasting blood samples were taken. During the second period, interventions were switched.

### Coffee preparation

We used Douwe Egberts brand, coarsely ground coffee (Sara Lee Co, Utrecht, Netherlands), consisting of a blend of arabica and robusta beans. This is the most widely used coffee brand and blend in the Netherlands. We packaged the coffee in evacuated plastic bags in daily portions to preserve the coffee aroma. Volunteers put 39 g ground coffee into a 1-L cafetière coffee pot (Blokker, Amsterdam). Six hundred milliliters of boiling water was poured onto the grounds as described (8). Subjects then stirred the brew and after 5 min they pushed down the plunger to separate the brew from the grounds. This resulted in 500 mL cafetière coffee. The mean (±SD) cafestol concentration was 34 ± 3 mg/L and the kahweol concentration was 26 ± 1 mg/L. Two such 500-mL portions of coffee were prepared each day. We provided the volunteers with insulated flasks to store the coffee and advised them to drink it over the whole day. We considered it highly likely that the subjects were compliant because few would enjoy drinking a large amount of strong coffee all at once.

### Blood samples and assays

Fasting venous blood samples were collected on day 15 of each intervention period. All samples were coded to hide the identity and intervention group of the subjects to laboratory personnel. For each individual, the samples obtained in the 2 intervention periods were analyzed simultaneously in the same batch.

Serum cholesterol, triacylglycerols, and alanine aminotransferase were analyzed in the clinical laboratory of our hospital according to standard clinical chemical procedures at 37°C on a Hitachi 747 analyzer (Roche Boehringer Mannheim, Almere, Netherlands). EDTA-treated blood samples for total homocysteine analysis were immediately placed on ice until the plasma was separated by centrifugation. EDTA-treated plasma samples for homocysteine, vitamin B-12, and folate analysis were stored at –20°C until analyzed. Total homocysteine in plasma was determined by automated reduction with NaBH<sub>4</sub>/dithiothreitol and derivitized by Thiolyte (Calbiochem, La Jolla, CA) as described previously (10). The inter- and intraassay variation was <5%. Concentrations of folate and vitamin B-12 were determined by using ion-capture IMx (Abbott Labs, Abbott Park, IL) (11, 12).

EDTA-treated whole-blood samples for vitamin B-6 analysis were stored at –20°C until analyzed. The determination of vitamin B-6 as pyridoxal-5'-phosphate (PLP) was performed with HPLC by using postcolumn derivatization with semicarbazide to obtain PLP-semicarbazone (13).

### Statistics

Differences in plasma concentrations of homocysteine, vitamin B-12, and folate; serum concentrations of cholesterol and triacylglycerols; and whole-blood concentrations of vitamin B-6 between the end of the no-coffee period and the end of the coffee period were calculated per subject and analyzed by using a two-sided unpaired *t* test (SPSS Inc, Chicago). Correlations between indexes were evaluated with Pearson's linear correlation procedure.

## RESULTS

Sixty-four volunteers (31 men and 33 women) with a mean (±SD) age of 43 ± 11 y (range: 24–70 y) completed the study. Six volunteers dropped out: 1 subject because of palpitations and tremor during the first days of drinking the cafetière coffee and 5 subjects for reasons unrelated to the study. The characteristics of the 64 volunteers who completed the study are shown in **Table 1**.

**TABLE 1**  
Baseline characteristics of the subjects

Characteristic	Value
Subjects ( <i>n</i> )	
Men	31
Women	33
Smokers	16
Age (y)	43.4 ± 11.3 <sup>1</sup>
Age range (y)	24–70
Body mass index (kg/m <sup>2</sup> )	24.5 ± 3.7
Cholesterol (mmol/L)	5.0 ± 0.8
Triacylglycerol (mmol/L)	1.0 ± 0.6
Alanine aminotransferase (U/L)	13.0 ± 4.7
Creatinine (μmol/L)	86.8 ± 10.6

<sup>1</sup> $\bar{x} \pm SD$ .

**TABLE 2**

Mean dietary intakes of subjects during the coffee and no-coffee (placebo) periods<sup>1</sup>

	No-coffee period	Coffee period	Difference (95% CI of difference)
Energy (MJ/d)	10.1	10.1	0.1 (−0.5, 0.5)
Fat (g/d)	96	97	1 (−6, 9)
Protein (g/d)	85	93	8 <sup>2</sup> (2, 13)
Carbohydrates (g/d)	278	270	−8 (−23, 7)
Fiber (g/d)	19	19	0 (−2, 1)
Fluid (g/d) <sup>3</sup>	2519	2880	361 <sup>2</sup> (195, 527)

<sup>1</sup>Three-day dietary-recall data were collected in both groups.

<sup>2</sup> $P < 0.05$ .

<sup>3</sup>Includes beverages as well as the water in solid foods.

In the coffee period, during which subjects drank 1 L coffee/d, significantly more fluid ( $\bar{x}$ : 361 g more) was consumed than during the no-coffee period (**Table 2**). The protein intake in the coffee period was 8 g higher than that in the no-coffee period. No significant correlation was seen between individual differences in plasma homocysteine concentrations and daily protein intakes between the 2 intervention periods ( $r = -0.03$ ,  $P = 0.83$ ). Other differences in reported dietary intakes were slight.

The mean concentration of plasma homocysteine was 12.8  $\mu\text{mol/L}$  at the end of the no-coffee period and 14.0  $\mu\text{mol/L}$  at the end of the coffee period (**Table 3**). We thus observed an increase in the homocysteine concentration of 10%, or 1.2  $\mu\text{mol/L}$ , caused by unfiltered cafetière coffee. Individual responses are shown in **Figure 1**. The effect of coffee on the homocysteine concentration was seen during both interventions and during each of the 3 shifts (Table 3). As expected (8), consumption of unfiltered coffee increased mean serum cholesterol by 10% (or 0.5 mmol/L), serum triacylglycerols by 36% (or 0.4 mmol/L), and serum alanine aminotransferase activity by 2.5 U/L (95% CI: 1.0, 3.9).

In 3 of the subjects, the alanine aminotransferase activity exceeded the upper limit of normal after 2 wk of coffee consumption. In one subject, this was observed at the end of the no-coffee

period. Alanine aminotransferase activity returned to normal within 2–3 wk in all but one subject, in whom values normalized only after 9 mo (data not shown). Coffee consumption did not significantly affect vitamin B-12 or folate concentrations in plasma (Table 3). The whole-blood vitamin B-6 concentration decreased by 21% (or 11.2 nmol/L) in the coffee period.

## DISCUSSION

We found that fasting plasma homocysteine concentrations increased by 10% in subjects with initially normal homocysteine concentrations who drank 1 L unfiltered coffee for 2 wk. The crossover design of our study and the distribution of subjects over 3 shifts eliminated chance fluctuations and seasonal influences as confounding factors (Table 3).

The dietary recalls, which focused on macronutrient intakes, showed that the subjects' diets remained essentially the same between the coffee and no-coffee periods, except for protein and fluid intakes—which were significantly higher during the coffee period. Recently, an inverse dose-response relation between dietary protein intake and serum homocysteine concentration was reported (6). On the basis of these data, the slight increase in protein intake we found in our study would only diminish the effect of coffee on homocysteine. However, homocysteine is formed from methionine (14) and an increased intake of methionine from dietary protein might increase homocysteine concentrations. Our volunteers had 8 g more protein in their diet during the coffee period (Table 2), which corresponds with 1–2 mmol methionine/d. However, even a variation in daily methionine intake of 4.2–31.2 mmol/d does not significantly affect fasting concentrations of homocysteine (15). There was also no correlation between the individual changes in plasma homocysteine concentration and in dietary protein intake. Thus, the difference in dietary protein intake did not explain the increase in homocysteine in our study. The difference in fluid intake was due to the amount of coffee people had to drink. They were not obliged to drink 1 L of alternative beverages in the no-coffee period. To our knowledge, plasma homocysteine is not affected by fluid intake; therefore, we conclude that the increase in homocysteine

**TABLE 3**

Plasma homocysteine and vitamin concentrations and serum lipids in subjects during the coffee and no-coffee (placebo) periods

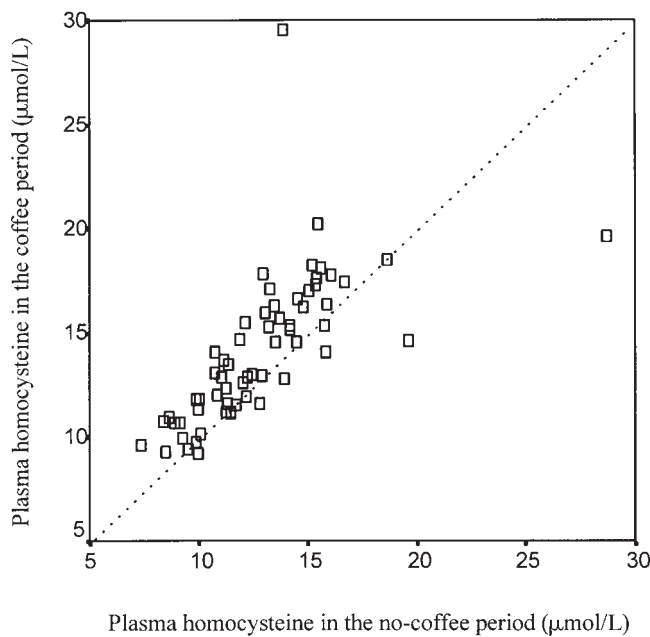
	No-coffee period <sup>1</sup>	Coffee period <sup>1</sup>	Difference	(95% CI of difference)
Plasma homocysteine ( $\mu\text{mol/L}$ )				
All ( $n = 64$ )	12.8 $\pm$ 3.3	14.0 $\pm$ 3.5	1.2 <sup>2</sup>	(0.5, 1.9)
Coffee first ( $n = 30$ )	12.5 $\pm$ 2.2	14.0 $\pm$ 2.6	1.5	(1.1, 2.0)
Placebo first ( $n = 34$ )	13.1 $\pm$ 4.0	14.0 $\pm$ 4.2	0.9	(−0.4, 2.2)
April shift ( $n = 27$ )	14.2 $\pm$ 3.9	15.6 $\pm$ 3.8	1.4	(−0.1, 3.0)
June shift ( $n = 17$ )	11.3 $\pm$ 1.8	12.3 $\pm$ 2.4	1.0	(0.1, 1.9)
September shift ( $n = 20$ )	12.2 $\pm$ 2.7	13.3 $\pm$ 2.9	1.2	(0.1, 1.8)
Serum lipids (mmol/L)				
Cholesterol ( $n = 64$ )	4.8 $\pm$ 0.8	5.3 $\pm$ 0.8	0.5 <sup>3</sup>	(0.3, 0.6)
Triacylglycerol ( $n = 64$ )	1.0 $\pm$ 0.5	1.4 $\pm$ 0.6	0.4 <sup>3</sup>	(0.2, 0.5)
Vitamins (nmol/L)				
Vitamin B-12 ( $n = 63$ ) <sup>4</sup>	263 $\pm$ 114	250 $\pm$ 100	−13	(−24, 1)
Folate ( $n = 64$ )	10.4 $\pm$ 3.7	10.8 $\pm$ 4.6	0.4	(−0.4, 1.1)
Vitamin B-6 ( $n = 59$ )	53.3 $\pm$ 17.5	42.1 $\pm$ 12.5	−11.2 <sup>3</sup>	(−14.5, −7.8)

<sup>1</sup> $\bar{x} \pm \text{SD}$ .

<sup>2</sup> $P = 0.001$ .

<sup>3</sup> $P < 0.0001$ .

<sup>4</sup>pmol/L.



**FIGURE 1.** Comparison of individual changes in plasma homocysteine between the coffee period and the no-coffee period. In the coffee period, subjects drank 1 L cafetière coffee/d for 2 wk. The interrupted line represents the line of identity.

was probably caused by the coffee.


Intakes of vitamin B-12, folic acid, and vitamin B-6 may affect homocysteine concentrations (16, 17). Concentrations of vitamin B-12 and folate in plasma did not change between the intervention periods, but the vitamin B-6 concentration decreased during the coffee period (Table 3). It is unlikely that this was caused by dietary changes because dietary intake was nearly constant (Table 2) and none of our subjects took vitamin supplements. In addition, the crossover design with multiple shifts makes it unlikely that there were major differences in the intake of vitamin B-6 associated especially with the intake of coffee. Six volunteers had vitamin B-6 concentrations between 29 and 35 nmol/L in the no-coffee period, slightly below the normal range (35–107 nmol/L). The effect of coffee on homocysteine was unchanged after exclusion of these subjects. Thus, the effect appears to have been independent of vitamin B-6 status.

We speculate that the effect of coffee on vitamin B-6 concentrations in blood might be mediated by caffeine. The chemical structure of caffeine (1,3,7-trimethylxanthine) is comparable with that of theophylline (1,3-dimethylxanthine) (18), which is a vitamin B-6 antagonist (19, 20). However, the effect of changes in vitamin B-6 status on fasting homocysteine concentrations is controversial. Some population studies reported an inverse association between fasting plasma homocysteine and vitamin B-6 concentrations (21, 22). On the other hand, an isolated vitamin B-6 deficiency does not necessarily result in an increase in fasting plasma homocysteine concentrations in healthy individuals (20, 23, 24). Therefore, the decrease in blood vitamin B-6 concentration during the coffee period offers one, but not necessarily the only, explanation for the effect of coffee on plasma homocysteine.

One important question is whether the effect on plasma homo-

cysteine was caused by substances present only in unfiltered coffee or by substances that are also present in filtered coffee. The study by Nygard et al (5) suggested that heavy coffee drinking in Norway is associated with an increase of 2  $\mu\text{mol/L}$  in the homocysteine concentration. The authors claimed that 95% of their population consumed filtered coffee. However, the cholesterol concentration in subjects drinking >8 cups coffee/d was 0.5  $\mu\text{mol/L}$  higher than that in the coffee abstainers. This is exactly what would be expected if the subjects had drunk unfiltered coffee (8), which is known to raise cholesterol; filtered coffee has no known effect on lipids. Therefore, we question whether subjects in the Norwegian study were indeed drinking unfiltered coffee. The association between coffee consumption and plasma homocysteine could not be confirmed in participants in the Atherosclerosis Risk in Communities Study in the United States (7), most of whom probably consumed filtered coffee. The discrepancy between these 2 observational studies suggests that not all types of coffee have the same effect on plasma homocysteine concentrations.

The 10% rise in plasma homocysteine after the subjects in our study drank 6 large cups unfiltered coffee/d could increase cardiovascular disease risk by 10% if homocysteine is a causal factor (3). In patients with fasting homocysteine concentrations >100  $\mu\text{mol/L}$  because of a homozygous cystathionine  $\beta$ -synthase deficiency, effective treatment markedly reduced the vascular thromboembolic events associated with this disease (25–28). It is still unknown whether a reduction in moderately elevated homocysteine concentrations will also reduce the risk of cardiovascular disease. Randomized controlled trials are underway that may answer this question (29).

Coffee consumption in most populations varies from 1 to 7 cups/d (18, 30), which is less than the amount (6 cups) of strong coffee used in our study. Thus, our results only apply to heavy coffee drinkers. We conclude that high intakes of unfiltered cafetière coffee increased the plasma homocysteine concentrations of our subjects, who had normal initial homocysteine concentrations. A high intake of unfiltered, boiled coffee was reported previously to elevate cardiovascular disease risk (31). Our data suggest that this elevation in risk may be due not only to the effect of such coffee on serum cholesterol concentrations, but also to its effect on plasma homocysteine. The effect of filtered coffee on plasma homocysteine concentrations remains to be established. Meanwhile, the observed effect of unfiltered coffee on plasma homocysteine in the present study suggests that individuals at risk of cardiovascular diseases should not drink large amounts of unfiltered coffee. 

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