Abstention from filtered coffee reduces the concentrations of plasma homocysteine and serum cholesterol—a randomized controlled trial[1–3]

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ABSTRACT

Background: Elevated concentrations of plasma total homocysteine (tHcy) and serum total cholesterol are risk factors for ischemic heart disease (IHD). Previous studies showed that the consumption of very high doses of unfiltered coffee increases tHcy and total cholesterol.

Objective: A prospective intervention study was performed to assess the effects of coffee consumption on the concentrations of tHcy and total cholesterol by using doses and brewing methods common in southeastern Norway.

Design: The study was an unblinded, controlled trial with 191 healthy, nonsmoking, coffee-drinking volunteers aged 24–69 y randomly assigned to 3 groups who were asked to consume for 6 consecutive weeks no coffee, 1–3 cups ($\approx$175–525 mL)/d, or $\geq$4 cups($\approx$700 mL)/d prepared in the manner to which they were accustomed. Blood samples were drawn when the subjects were randomly assigned and at 3 and 6 wk of the trial. Dietary data were collected by questionnaire.

Results: Ninety-seven percent of the participants reported being regular consumers of caffeinated filtered coffee. Abstention from coffee for 6 wk was associated with a decrease in the tHcy concentration of 1.08 μmol/L and a decrease in the total cholesterol concentration of 0.28 mmol/L in participants who had been drinking on average 4 cups of filtered coffee daily for the past year. Adjustments for several possible confounders did not alter the results.

Conclusion: Abstention from filtered coffee in doses that are commonly consumed was associated with lower concentrations of tHcy and total cholesterol. *Am J Clin Nutr* 2001;74:302–7.

KEY WORDS Filtered coffee, homocysteine, cholesterol, folate, ischemic heart disease

INTRODUCTION

Coffee has attracted interest for a long time as a potential health hazard. Although early observations indicated a relation between the occurrence of ischemic heart disease (IHD) and coffee intake, later studies in different population groups showed inconsistent results (1–5). In the mortality follow-up of Norwegian men and women, Tverdal et al (4) showed that coffee consumption is strongly related to IHD mortality. Part of this association could be explained by a cholesterol-raising effect of terpenoids [which was found to be particularly pronounced in unfiltered coffee (6, 7)] or by cigarette smoking, but a significantly increased risk remained even after adjustment for serum total cholesterol and smoking. This seemingly unexplained association could either be a direct effect of coffee through unknown risk factors not adjusted for or could reflect an atherothrombogenic lifestyle among heavy coffee drinkers (8). The former explanation gained increased credibility because of the cross-sectional report from The Hordaland Homocysteine Study of a significant positive relation between coffee consumption and total homocysteine (tHcy) (9, 10) in $\geq$14 000 Norwegian consumers of filtered, unfiltered, and instant, but not decaffeinated, coffee (11). The association was significant even after adjustment for the intake of B vitamins and other nutritional habits that were expected to influence the concentration of tHcy.

A recent intervention study (12) showed that very high intake of unfiltered coffee (1 L/d) increased plasma tHcy by 10% (from 12.8 to 14.0 μmol/L). Although the authors were not able to conclude whether this association depends on the brewing method, it was suggested that unfiltered coffee is more likely to increase tHcy than is filtered coffee. It was speculated that the findings in The Hordaland Homocysteine Study with respect to both tHcy and total cholesterol might be explained by underreporting of consumption of unfiltered coffee.

To assess the coffee-tHcy as well as the coffee–total cholesterol association, we carried out a randomized intervention trial while controlling for both dietary habits and brewing methods. Because cigarette smoking has been associated with low folate concentrations and elevated tHcy concentrations, only nonsmokers were included.

TABLE 1
Baseline characteristics of the participants in the 3 groups[1]

Baseline characteristics	Coffee abstainers (n = 65)	1–3 cups/d (n = 61)	≥4 cups/d (n = 65)
Age (y)[2]	53 (28–69)	52 (25–70)	51 (24–69)
Women [n(%)]	44 (67.7)	33 (54.1)	32 (49.2)
BMI (kg/m^2)	25.8 ± 3.7	25.6 ± 2.7	24.6 ± 3.1
Blood pressure (mm Hg)	137/87	136/87	133/87
Total homocysteine (μmol/L)	9.9 ± 2.8	9.9 ± 2.4	9.5 ± 2.2
Serum folate (nmol/L)	13.2 ± 5.8	14.2 ± 10.1	12.7 ± 7.0
Whole-blood folate (nmol/L)	232 ± 118	209 ± 71	218 ± 80
Serum vitamin B-12 (pmol/L)	321 ± 111	341 ± 109	324 ± 84
Total cholesterol (mmol/L)	5.87 ± 0.95	5.69 ± 1.10	5.74 ± 1.03
HDL cholesterol (mmol/L)	1.52 ± 0.40	1.49 ± 0.34	1.42 ± 0.35
Triacylglycerol (mmol/L)	1.47 ± 0.82	1.39 ± 0.70	1.41 ± 0.63
Lipoprotein(a) (mmol/L)	38.1 ± 43.5	27.0 ± 32.3	38.2 ± 46.2

[1] $\overline{x}$ ± SD. There were no significant differences between the groups.
[2] Median; range in parentheses.

SUBJECTS AND METHODS

Trial design and sample size calculation

The study was organized as a prospective, unblinded controlled trial with the participants randomly assigned to 3 different treatment groups that were to consume for 6 consecutive weeks no coffee, 1–3 cups (≈175–525 mL) of coffee/d, or ≥4 cups (≈700 mL) of coffee/d. On the basis of the differences observed in the cross-sectional study (11), we estimated the expected difference in tHcy between the 2 extreme groups to be on the order of 1.5 μmol/L. Given a statistical power of 0.9 (90% probability for detecting this difference if it is there) at a significance level of 0.05, each group would need 60 persons.

Subjects and procedure

The participants were recruited by an advertisement we placed in the largest newspaper in Oslo. Inclusion criteria were age 24–69 y, a history of daily consumption of coffee for ≥5 y, and no daily tobacco smoking for the past 6 mo. All participants gave written consent to participation after they were provided with oral and written information; on entering the trial, they completed a questionnaire relating to exclusion criteria. Approval for the study was obtained from the Regional Research Ethics Committee. Exclusion criteria included clinically recognized chronic diseases, such as cardiovascular diseases, cancer, renal disorders, liver disease, diabetes mellitus; the use of antiepileptic or cholesterol-lowering drugs; recognized vitamin deficiency in the past; and serum folate concentrations below the lower value of the reference range (6–24 nmol/L). All participants were asked to follow their usual diet during the trial: the coffee-consuming groups were permitted to drink the type of coffee to which they were accustomed. Body weight and blood pressure were monitored throughout the 6-wk period in the 3 intervention groups. Each participant was seen at 3 visits that were spaced 3 wk apart. Dietary habits were recorded at the start and end of the trial.

Nonfasting blood samples were drawn at the time of random assignment and 3 and 6 wk after the start of the trial. Plasma was prepared from EDTA-treated blood within 2 h after storage at 4 °C, and blood serum was prepared without additives after storage at room temperature. Before analysis, plasma and serum were stored at −20 °C, and blood for the whole blood folate analysis was stored at −70 °C. The tHcy, folate, and lipid concentrations were

determined within 3 mo, and vitamin B-12 concentrations were determined 7 mo after the trial was finished. All 3 samples from each patient were always analyzed consecutively in the same run.

Effect variables

Plasma tHcy was measured by using the Abbott IMX homocysteine assay (Abbott Laboratories, Abbott Park, IL; CV: 4%) and the lipids in serum [HDL cholesterol, triacylglycerol, LDL cholesterol, and lipoprotein(a)] were measured by the routine methods used in our hospital. Total cholesterol was measured by an enzymatic assay analyzed on a Cobas Integra instrument (Roche, Basel, Switzerland; CV: 3%). Serum folate in whole blood and vitamin B-12 were measured with an AutoDelphia instrument (Wallac Oy, Turku, Finland; CV: 4.5%). The analytic principle for the folate assays was based on the measurement of time-resolved fluorescence from a solid-phase immunoassay with a competitive reaction between europium-labeled pteroyl-glutamic acid and sample folate for a limited amount of folate-binding protein. The analytic principle for the vitamin B-12 fluoroimmunoassay was based on a competitive reaction between europium-labeled vitamin B-12 and sample vitamin B-12 for a limited amount of binding sites on intrinsic factor. The effects on the biochemical variables were assessed as the differences between the first and the last measurement within each group, as well as the differences between the changes within the groups. The dietary habits were assessed at the beginning and end of the trial with a validated Norwegian food-frequency questionnaire (13, 14). After the intervention period, the participants were asked about their self-perceived changes in the intake of important foods and drinks. Height, weight, and blood pressure were recorded at the beginning of the trial and after 6 wk.

Statistical methods

Statistical analyses were carried out by using the computer program SPSS version 9.0 for WINDOWS (SPSS Inc, Chicago). The paired t test was used for the comparison of the differences of the biochemical variables taken between the start of the trial and after 6 wk. To adjust for possible confounding effects on the differences of tHcy and total cholesterol, analysis of covariance
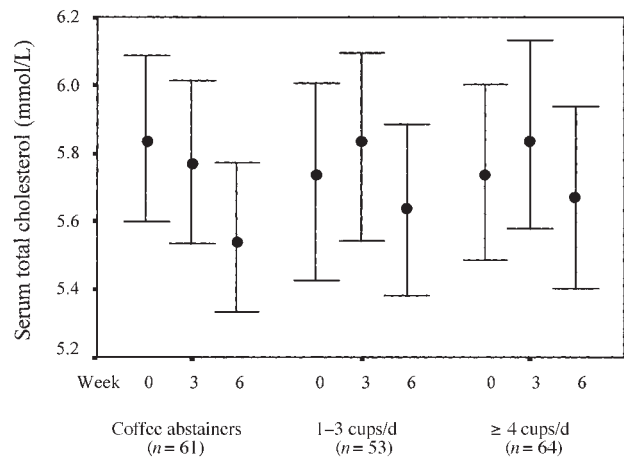
FIGURE 1. The mean (± SD) concentrations of serum total cholesterol in the 3 treatment groups 0, 3, and 6 wk after the start of the trial.
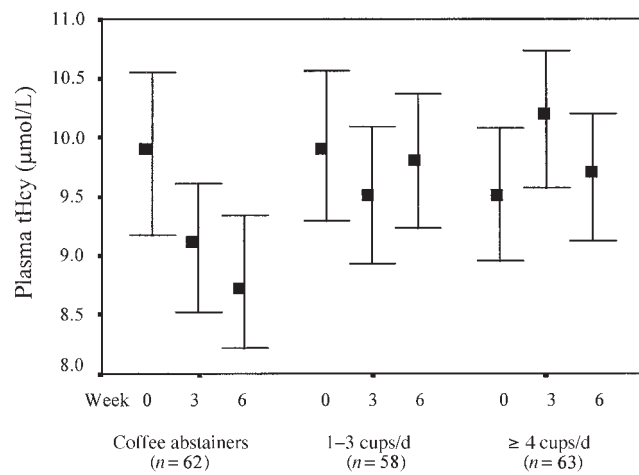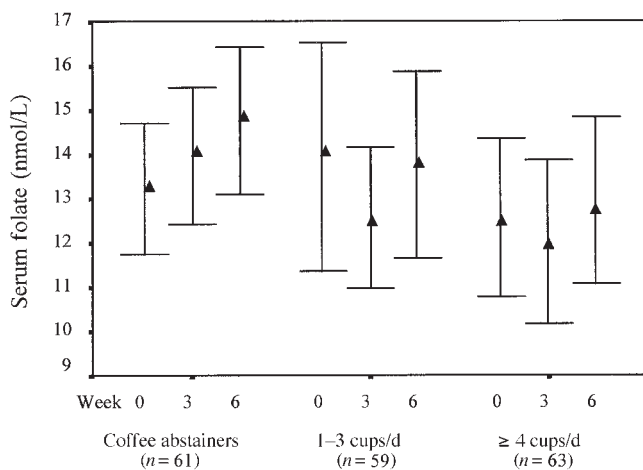
FIGURE 2. The mean (±SD) concentrations of plasma total homo-cysteine (tHcy) in the 3 treatment groups 0, 3, and 6 wk after the start of the trial.

as well as multiple linear regression analyses with sex, age, change in body mass index (BMI), alteration in folate concentrations, and self-reported intake of tea and fruit juice were carried out. Statistical significance was $P < 0.05$.

RESULTS

Two hundred fourteen ostensibly healthy persons were recruited; 23 were excluded because of low serum folate concentrations (<6 nmol/L), which left a total of 191 participants. Two of these did not come to their scheduled visits, and 4 decided to withdraw from the trial. Ninety-seven percent of the participants had been consuming filtered brewed, caffeinated coffee habitually before the study, and the average amount consumed was 4.9 cups/d. The questionnaire to assess adherence to the group assignment and possible changes in diet during the trial was completed by 182 (95%) of the participants.

FIGURE 3. The mean (±SD) concentrations of serum folate in the 3 treatment groups 0, 3, and 6 wk after the start of the trial.

TABLE 2
Effect of coffee on the concentration of serum total cholesterol (TC) determined by multiple linear regression analysis[1]

	Difference in serum TC from coffee abstainers	
Adjustment variables	1–3 cups/d	≥4 cups/d
	mmol/L	
Sex and age	0.213 (0.066)	0.217 (0.035)
Sex, age, and change in BMI	0.240 (0.063)	0.222 (0.043)
Plus change in serum folate	0.224 (0.086)	0.233 (0.036)
Plus change in whole-blood folate	0.236 (0.068)	0.217 (0.046)
Plus change in total homocysteine	0.220 (0.120)	0.028 (0.825)
Plus change in tea intake	0.242 (0.085)	0.349 (0.012)
Plus change in fruit juice intake	0.257 (0.050)	0.203 (0.078)

[1] Mean difference in the change in serum TC (week 6 – week 0). *P* values in parentheses.

The baseline characteristics of the eligible participants according to treatment group are shown in **Table 1**. There was a slightly higher proportion of female participants among the coffee abstainers, but there were no other significant differences between groups.

The effects of the intervention on total cholesterol, tHcy, and serum folate are shown in **Figures 1–3**. The abstention from coffee drinking for 6 wk was associated with an average decrease in total cholesterol of 0.28 mmol/L (95% CI: 0.13, 0.42) and a decrease in tHcy of 1.08 μmol/L (95% CI: 0.71, 1.44) compared with the initial concentration. The concentration of serum folate in the same group increased by 1.57 nmol/L after 6 wk (95% CI: 0.01, 3.11; $P = 0.048$). In the 2 groups that drank coffee, the changes in total cholesterol, tHcy, and serum folate concentrations were not significant after 6 wk. The concentration of whole blood folate decreased after 3 wk and increased after 6 wk in all 3 groups, but no significant trends were observed. The concentration of vitamin B-12 in serum did not change in any of the groups (data not shown).

The average differences of the 3 groups in the changes in (linear logistic regression analysis) total cholesterol, tHcy, and folate concentrations from week 0 to week 6 are shown in **Tables 2–4**. The results after adjustments for possible confounding variables are also shown. The difference between the average change in total cholesterol from week 0 to week 6 when comparing the group con-

TABLE 3
Effect of coffee on the concentration of plasma total homocysteine (tHcy) in plasma determined by multiple linear regression analysis[1]

	Difference in plasma tHcy from coffee abstainers	
Adjustment variables	1–3 cups/d	≥4 cups/d
	mmol/L	
Sex and age	1.05	1.29
Sex, age, and change in BMI	1.31	1.57
Plus change in serum folate	1.35	1.54
Plus change in whole-blood folate	1.31	1.56
Plus change in total cholesterol	1.22	1.43
Plus change in tea intake	1.35	1.73
Plus change in fruit juice intake	1.26	1.52

[1] Mean difference in the change in plasma tHcy (week 6 – week 0). All values were significantly different from coffee abstainers, *P* < 0.001.

TABLE 4
Effect of coffee on the concentration of serum folate determined by multiple linear regression analysis[1]

Adjustment variables	Difference in serum folate from coffee abstainers	
	1–3 cups/d	≥4 cups/d
	nmol/L	
Sex and age	−1.61	−1.00
Sex, age, and change in BMI	−2.35	−0.21
Plus change in whole-blood folate	−2.47	−1.23
Plus change in total homocysteine	−3.01	−0.42
Plus change in total cholesterol	−2.22	−1.42
Plus change in tea intake	−1.87	−1.30
Plus change in fruit juice intake	−2.22	−1.15

[1] Mean difference in the change in serum folate (week 6 – week 0). None of the differences were significant.

suming ≥4 cups of coffee/d with the group that abstained from coffee was significant after adjustments for most of the confounders except for tHcy. The latter may be explained by the positive correlation observed between the decreases in the concentrations of tHcy and total cholesterol in coffee abstainers. Adjustments for sex, age, BMI, serum folate, whole blood folate, total cholesterol, or a self-reported change in the consumption of tea or fruit juice during the trial did not alter the finding of a strong association between the consumption of coffee and the concentration of tHcy. The adjusted differences between the changes in tHcy concentration from week 0 to week 6 were consistently larger between the coffee abstainers and the group with the highest coffee consumption than between the abstainers and the in-between group (1–3 cups/d). The average difference between the changes in serum folate in the 3 groups was not significant. No significant correlation was observed between the changes in tHcy concentrations and the changes in folate concentrations between the end and start of the trial.

Participants who either increased or decreased their habitual coffee consumption reported changes in the intake of other fluids during the intervention: primarily, tea, juice, and water. In the linear logistic regression analyses, adjustments for the intake of tea and juice were carried out because of the folate content of these beverages. The adjustments did not alter the results (Tables 2–4). Reported changes in intake of fruits and vegetables did not differ significantly among the groups (data not shown), but significant positive associations with changes in coffee consumption were registered for the intake of sweets and cakes. Adjustment for brewing methods did not alter the results (data not shown). As shown in **Tables 5** and **6**, analyses of covariance produced similar findings.

DISCUSSION

This randomized controlled trial confirmed the previous cross-sectional findings (10) of an association between the consumption of filtered coffee and concentrations of total cholesterol and tHcy. In this study, abstention from filtered coffee was associated with a decline in tHcy of >1 µmol/L (10%) in daily coffee drinkers. This closely corresponds to the differences observed between coffee abstainers and those drinking 5–8 cups of filtered coffee per day in The Hordaland Homocysteine Study. The relation was graded with lower tHcy concentrations corresponding to lower numbers of cups of coffee consumed.

Our results are also in line with the recently published findings of increased plasma tHcy after daily consumption of 1 L unfiltered coffee (12). The fact that the magnitude of the effect on tHcy was similar in our study with filtered coffee indicates that this effect is caused by compounds that are not removed in the filtering process.

The effect of coffee on tHcy could be mediated directly or indirectly by the metabolism of the B vitamins folate, vitamin B-6, or vitamin B-12. Of these, the concentration of serum folate is known to be the strongest determinant of the concentration of plasma tHcy, whereas the effects of vitamins B-6 and B-12 are considered

TABLE 5
Effect of coffee on the concentration of serum total cholesterol TC determined by analysis of covariance[1]

Coffee intake group	TC		Difference (week 6 – week 0)		
	Week 0	Week 6	Unadjusted	Adjusted[2]	P
Coffee abstainers	5.87	5.59	−0.27	−1.27	—
1–3 cups/d	5.69	5.71	0.02	−0.07	0.078
≥4 cups/d	5.74	5.67	−0.07	−0.06	0.061

[1] $\bar{x}$.
[2] Adjusted for sex, age, and BMI at baseline.

TABLE 6
Effect of coffee on the concentration of plasma total homocysteine (tHcy) determined by analysis of covariance[1]

Coffee intake group	tHcy		Difference (week 6 – week 0)			
	Week 0	Week 6	Unadjusted	Adjusted[2]	Adjusted[3]	Adjusted[4]
Coffee abstainers	9.90	8.74	−1.16	−1.30	−1.27	−1.16
1–3 cups/d	9.89	9.75	−0.14[5]	−0.12[5]	−0.09[5]	−0.15[5]
≥4 cups/d	9.48	9.69	0.22[5]	0.17[5]	0.20[5]	0.17[5]

[1] $\bar{x}$.
[2] Adjusted for sex, age, and BMI.
[3] Adjusted for serum folate.
[4] Adjusted for whole-blood folate.
[5] Significantly different from coffee abstainers, $P < 0.0001$.

to be less important (15). Indeed, the concentration of vitamin B-12 was not influenced by coffee consumption in the present study.

The effect of coffee on whole blood folate was not consistent. The reason may be the short trial period, which lasted an average of only 42 d. The concentration of folate in red blood cells or in whole blood is a measure of intracellular folate status and reflects the folate status during the past 120 d (16).

There was an increased serum folate concentration in the coffee abstainers, but the differences in the folate changes between the groups were not statistically significant. These results cannot support or reject the hypothesis that coffee drinkers have different intakes of folate or that coffee interferes with the uptake of folate from nutrients. The lack of a relation between the effect of coffee on tHcy and an alteration in the concentration of folate is in line with the recent findings of Grubben et al (12).

Dietary trials with subjects consuming a free diet frequently create interpretation problems caused by the need for a constant intake of energy and fluid. Any induced change in the sources of these 2 variables is likely to be compensated for by changes in other sources. This will be of no importance as long as the replacements are inert with regard to the outcome factors. In the present experiment, monitoring of body weight did not indicate any systematic changes in energy intake, but the participants who changed their coffee-drinking habits reported that they compensated for the changes in fluid intake by increasing or decreasing the intake of other beverages. The most common replacements of coffee in this study were black tea and fruit juice. Both drinks have been reported to affect the intake of folate (17), but adjustment for these dietary changes did not change the main results in the trial. This is also in line with the results from The Hordaland Homocysteine Study, in which crude analyses showed that the consumption of black tea was negatively related to tHcy concentrations in coffee drinkers, whereas no significant relation was seen in coffee abstainers. This correlation disappeared after adjustment for smoking habits and vitamin consumption. The coffee-tHcy relation in the present trial, however, remained remarkably stable even after adjustment for all the variables addressed in the study, suggesting a robust association. The possibility that other confounding factors might have influenced the results cannot be ruled out; however, such an influence would have to have been strong if this were the case.

Surprisingly, the effect of coffee on total cholesterol was more pronounced than in the results of previous studies (18). The observation of a decrease in total cholesterol by 0.28 mmol/L in the present study indicates that the terpenoids that cause an elevated concentration of total cholesterol are only partly removed by a coffee filter.

The present study only addresses the issue of a causal relation between coffee intake and the concentrations of some biochemical variables, but the results can be used to assess the possible effects of coffee on IHD risk. The average alteration in tHcy per cup of coffee consumed before the trial was estimated to be ≈ 0.22 μmol/L. An increase of 5 μmol tHcy/L is associated with an odds ratio for myocardial infarction of 1.57 (19). This odds ratio would correspond to an increase in coffee consumption of 23 cups of coffee/d. Abstaining from a previous daily consumption of 4 cups of filtered coffee might therefore reduce the homocysteine-attributed risk of IHD by $\approx 10\%$. The Dutch study (12) showed a similar IHD risk with consumption of 6 cups of unfiltered coffee. Abstaining from 4 cups/d would also reduce the concentration of total cholesterol by 0.28 mmol/L and reduce the total cholesterol–attributed risk of IHD by 15% (20).

We conclude that abstaining from even commonly consumed amounts of filtered coffee may lower the concentrations of both tHcy and total cholesterol. It is not obvious that the effect on tHcy is related to alterations in folate status. If there is interference with folate, it may theoretically happen by 3 different mechanisms. First, coffee drinkers could have lower intake of folate-containing food. Second, the intake of coffee could interfere with the absorption of folate from the diet. Third, there might be substances in coffee that interfere with folate-homocysteine metabolism. ☘

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