



Original Contribution

The effects of coffee consumption on lipid peroxidation and plasma total homocysteine concentrations: a clinical trial[☆]

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Abstract

Despite extensive research, the cardiovascular effects of coffee consumption in humans remain controversial. Our aim was to investigate the excretion of coffee phenols and the effects of filtered coffee consumption on oxidative stress and plasma homocysteine (tHcy) concentration in humans. The study consisted of a multiple-dose clinical supplementation trial and a single-dose study. In the long-term trial, 43 healthy nonsmoking men optionally consumed daily either no coffee, 3 cups (450 mL), or 6 cups (900 mL) of filtered coffee for 3 weeks, while in the short-term study 35 subjects consumed a single dose of 0, 1 (150 mL), or 2 cups (300 mL) of coffee. Long-term consumption of coffee increased the urinary excretion of caffeic and ferulic acid. The change in the total excretion of phenolic acids in 3 and 6 cups groups represented 3.8 and 2.5% of the amount ingested daily. Plasma tHcy concentrations increased nonsignificantly, but the consumption of coffee had neither short- nor long-term effects on lipid peroxidation or the activity of measured antioxidant enzymes. In conclusion, the consumption of filtered coffee does not have any detectable effects on lipid peroxidation in healthy nonsmoking men. The effect of coffee consumption on tHcy concentrations needs further investigation.

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Keywords: Coffee; Phenols; Lipid peroxidation; Plasma total homocysteine; Humans; Free radicals

Introduction

Coffee drinking is among the most widespread habits in the world and thus its effects on health may be highly relevant for public health. During the past three decades, a wealth of epidemiological studies addressing the role of coffee drinking in coronary heart disease (CHD) etiology has been published with controversial results from different populations [1]. Coffee drinking is one of many behavioral traits that are associated with each other, making it difficult to differentiate the effects of coffee from other dietary and behavioral factors.

The current opinion is that the oxidative modification of LDL plays an important role in the pathogenesis of atherosclerosis [2–4]. Recently, coffee has aroused scien-

Abbreviations: ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; BMI, body mass index; CHD, coronary heart disease; γ -GT, gamma-glutamyltransferase; GPX, plasma glutathione peroxidase activity; HCL, hydrochloric acid; MeOH, methanol; PBS, phosphate-buffered saline; PON, serum paraoxonase; tHcy, total homocysteine; TRAP, total peroxy radical trapping potential.

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tific interest because it is a rich source of a number of phenolic compounds with antioxidant effects *in vitro* [5,6]. Main polyphenols in coffee are phenolic acids such as chlorogenic and caffeic acid. Caffeine and its metabolites, di- and monomethylxanthines, have also been shown to have antioxidative properties [7]. Recent studies also suggest that coffee phenols are absorbed and ingestion of single dose of coffee may have antioxidative effects in humans [8–10].

In addition to the theoretical beneficial effects, coffee has recently aroused interest also because in several supplementation studies the consumption of coffee has increased the concentration of plasma total homocysteine (tHcy) in humans [11–13]. Elevated plasma tHcy concentrations have been associated with increased lipid peroxidation [14] and it is also suggested to be an independent risk factor for cardiovascular disease [15]. Chlorogenic acid and caffeine have been identified to be mainly responsible for the increase of tHcy after coffee consumption [11,12].

To this date there are only scarce data about the systemic availability of coffee phenols, their antioxidant effects, and the effects of coffee consumption on plasma tHcy concentrations in humans. The aim of the present study was to study the short- and long-term effects of coffee consumption on serum lipid peroxidation, on the activity of antioxidant enzymes, and on plasma tHcy concentrations in healthy men.

Materials and methods

Subjects

Forty-five nonsmoking volunteer men with a mean age of 26 ± 6 years were recruited from the Kuopio area in eastern Finland through advertisement via e-mail and intranet in the University of Kuopio. Potential participants were screened in an interview for the following eligibility criteria: no severe obesity (Body Mass Index, BMI < 32 kg/m²), no regular use of any drugs or supplements with antioxidative or lipid-lowering properties, no chronic diseases such as diabetes, CHD, claudication, cerebrovascular disease, hypothyroidism, or other major illness, willingness to abstain from coffee drinking or to consume 3 or 6 cups of coffee for 3 weeks. Of these subjects, 35 men participated also in the short-term study of the acute effects of coffee drinking in which they consumed a single dose of 0, 1, or 2 cups of coffee. To enhance compliance, volunteers were given the choice to participate in one or both of the studies. All of the mentioned criteria were ascertained prior to study entry. Written informed consent was obtained in writing from all participants after they had read a description of the experimental procedures. The study protocol was approved by the Research Ethics Committee, Hospital District of Northern Savo.

Study design

Long-term study

A 2-week run-in period preceded the long-term study and during this period the use of coffee, tea, red wine, cocoa, and chocolate was forbidden. In addition the intake of fruit- and berry-derived juices was restricted to a maximum of 2 glasses (300 mL) per day. Subjects were given caffeine tablets to be used if necessary to deal with withdrawal symptoms. The maximum daily amount of caffeine was the amount comparable to that obtained from the daily study bolus in the long-term study (0, 300, or 600 mg). After the run-in period subjects were free to choose whether they wanted to consume 0, 3 (450 mL), or 6 cups (900 mL) of coffee during the 3-week supplementation period. In order to ensure compliance, the study was not randomized. The ingested amounts of phenolic acids through coffee were 0, 364, and 728 mg/d in the 0, 3, and 6 cup groups, corresponding to molar amounts of 0, 1122, and 2243 $\mu\text{mol/day}$.

The subjects were advised to avoid the use of alcohol and analgesics 3 days and vigorous physical activity 1 day before the study visits. The subjects collected a 24-h urine sample before study visits and blood samples were drawn with Venoject vacuum tubes (Terumo) after an overnight fast (10 h). A 4-day food record was collected before and during the last week of the intervention period to control for possible confounding factors and to check compliance with the dietary instructions. The instructions for the food records were given, checked with the subjects, and analyzed by a nutritionist using Nutrica software, Social Insurance Institution, Helsinki, Finland (version 2.5).

The coffee used in this study was finely ground coffee packed in 500 g packages. Subjects were instructed to measure the daily amount of coffee (7–8 g of grounds per one 150-mL cup), prepare the coffee by filtering through paper, and to consume the total daily amount in three separate portions. The coffee used in the study was delivered by Oy Paulig Ab, Helsinki, Finland.

Short-term study

The short-term study was conducted directly after obtaining the blood samples for the supplementation period of the long-term study. The subjects remained in the same group as in the long-term study, but consumed a single dose of 1/3 of the total daily dose consumed in the long-term study (0, 1, or 2 cups, 0, 150, or 300 mL, respectively). They continued to fast (intake of tap water was allowed). A blood sample was taken 1.5 h after coffee ingestion. From the blood samples, the following markers of lipid peroxidation were analyzed: concentration of serum LDL-conjugated dienes, plasma hydroxy fatty acids, and plasma F₂-isoprostanes.

Resistance of serum lipids to oxidation

The resistance of serum lipids to oxidation was measured as described previously [16]. Briefly, serum was diluted to a

concentration of 0.67% in 0.02 mol/L phosphate-buffered saline (PBS), pH 7.4. Oxidation was initiated by addition of 100 μ l of 1 mmol/L CuCl₂ into 2 mL of diluted, prewarmed (30°C) serum. The formation of conjugated dienes was followed by monitoring the change in 234 nm absorbance at 30°C on a Beckman DU-6401 spectrophotometer (Fullerton, CA) equipped with a six-position automatic sample changer. The change in absorbance was recorded every 5 min for 4 h. The time required from the start to the maximal rate of the reaction (lag time) was determined.

Serum LDL-conjugated dienes

The oxidation of LDL in vivo was assessed as the amount of conjugated dienes as described previously [17]. In brief, serum LDL was isolated by precipitation with buffered heparin. The precipitate was resuspended in PBS. Cholesterol concentration was determined and the rest of the suspension was used for conjugated diene measurement. Lipids were extracted from the LDL by mixture of chloroform and methanol (3:1), dried under nitrogen, and redissolved in cyclohexane, and the amount of conjugated dienes was measured spectrophotometrically at 234 and 300 nm. Absorbance at 300 nm was subtracted from that at 234 nm. The conjugated diene concentration was calculated per cholesterol concentration in LDL.

Plasma hydroxy fatty acids

Plasma C18 hydroxy fatty acids were measured by gas chromatography/mass spectrometry (GC/MS) method [18]. Plasma fatty acids and fatty acid hydroperoxides were first stabilized by hydrogenation using platinum as a catalyst, saponified, esterified by diazomethane, and finally, to separate plasma hydroxy fatty acids from fatty acids, extracted by solid-phase minicolumns. Prior to analysis, hydroxy groups were methylated with tetramethylammonium hydroxide. Concentrations of different methoxy monohydroxy fatty acid methyl esters were determined by electron impact mass spectrometry. Monohydroxy fatty acids C17 and C19 were used as internal standards.

Plasma F₂-isoprostanes

A deuterated prostaglandin F_{2 α} internal standard was added to plasma, and F₂-isoprostanes were extracted with C₁₈ and silica minicolumns. Compounds were converted to pentafluorobenzyl ester trimethylsilylether derivatives and analyzed by a GC/MS assay [19].

Activity of antioxidant enzymes

Plasma glutathione peroxidase activity (GPX) was determined by a commercial kit (Ransel RS 505, Randox Laboratories, San Diego, CA) by using Konelab 20 Analyzer (Thermo Clinical LabSystems, Vantaa, Finland). Serum paraoxonase (PON) activity was measured from serum based on its capacity to hydrolyze paraoxon. The formation of *p*-nitrophenol was monitored at 405 nm in Tris-HCl buffer, pH 8.0, in the presence of Ca²⁺ [20].

Plasma total homocysteine

Plasma tHcy concentration was analyzed by high performance liquid chromatography (HPLC) at the National Public Health Institute, Helsinki, Finland, as described previously [21]. The results represent total plasma homocysteine and are referred to as plasma tHcy. The coefficients of variation (CV) between batches ($n = 30$) for two pooled plasma samples were 5.7% (7.3 μ mol/L) and 7.1% (10.5 μ mol/L).

Other measurements

Serum cholesterol (Konelab, Espoo, Finland) and triacylglycerides (Roche Diagnostics, Mannheim, Germany) were determined with enzymatic colorimetric tests. Serum LDL cholesterol was determined with a direct measurement without precipitation (Konelab). Serum HDL cholesterol was measured from the supernatant after magnesium chloride dextran sulfate precipitation. Blood cell profile, including erythrocyte, leukocyte, and thrombocyte counts and hemoglobin, was measured by a blood cell counter (Advia 60, Bayer, Tarrytown, NY). Serum aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) were measured with a clinical chemistry analyzer (Konelab). The activity of serum gamma-glutamyltransferase (γ -GT) was measured with the International Federation of Clinical Chemistry method [22]. Serum fatty acids were analyzed after extraction with chloroform–methanol and methylation with sulphuric acid–methanol by a gas chromatograph (Hewlett Packard 5890, Avondale, PA) equipped with a flame ionization detector and a NB-351 capillary column (HNU-Nordion, Helsinki, Finland). Plasma folate and vitamin B₁₂ were measured simultaneously by radioimmunoassay (Quantaphase II, Bio-Rad, Hercules, CA).

Phenolic acid analyses of coffee and urine

Phenolic acid analyses were carried out with HPLC using a coulometric electrode array detector. Compounds were separated with gradient elution using end-capped C 18 column Inertsil ODS-3 (150 \times 3 mm) packed with 3- μ m particles and C 18 guard column (10 \times 3 mm, 5 μ m particles). Mobile phase consisted of eluent (A) 50 mM KH₂PO₄/H₃PO₄ buffer pH 2.3:MeOH 90:10 (v/v) and (B) 50 mM KH₂PO₄/H₃PO₄ buffer pH 2.3:MeOH:ACN 40:40:20 (v/v/v). Chlorogenic, caffeic, ferulic, *p*-, *m*-, *o*-coumaric, sinapinic, protocatechuic, and gallic acid were measured from coffee and from urine; additionally two metabolites 3,4-dihydroxyphenyl and *m*-hydroxyphenylacetic acids were measured. Coffee analysis was carried out after the combination of the enzyme and base hydrolysis. Twenty-four-hour urine samples were hydrolyzed with β -glucuronidase and sulfatase obtained from *Helix pomatia*. Hydrolyzed samples were extracted with diethylether, evaporated under N₂ flow, and dissolved in MeOH. Samples were diluted with eluent prior to HPLC run.

The study coffee contained 80.9 \pm 3.3 mg/100 mL of phenolic acids; the major compound was chlorogenic acid,

Table 1
Baseline characteristics

	Daily coffee intake			<i>P</i> (ANOVA)*
	0 cups (<i>n</i> = 15)	3 cups (<i>n</i> = 14)	6 cups (<i>n</i> = 14)	
Age (years)	23.7 ± 3.8	25.4 ± 6.0	29.7 ± 6.8	0.020
BMI (kg/m ²)	23.5 ± 3.6	22.8 ± 3.5	26.1 ± 3.4	0.042
Total cholesterol (mmol/L)	4.1 ± 1.0	4.2 ± 0.7	4.7 ± 0.7	0.148
ASAT (U/L)	22.8 ± 6.1	24.1 ± 6.3	45.7 ± 61.1	0.163
ALAT (U/L)	26.1 ± 11.0	26.7 ± 16.9	51.1 ± 42.8	0.027
γ-GT (U/L)	17.5 ± 7.0	16.9 ± 6.4	27.9 ± 12.4	0.003

ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; γ-GT, gamma-glutamyltransferase.

* *P* for the differences in changes between the groups (one-way ANOVA).

representing approximately 90% of the quantified phenolic acid total. Another abundant phenolic acid was ferulic acid representing 8% of the quantified phenolic acids. Small amounts of *p*-coumaric, caffeic, protocatechuic, and sinapinic acid were also detected.

Statistical analyses

Means between study groups at baseline were compared by analysis of variance (ANOVA). The changes of means between study groups were compared by multivariate analysis of variance (MANOVA). Because of the difference in the age and BMI between study groups in the baseline, age, BMI, and baseline values of the parameter tested were used as covariates. The post hoc Tukey test was used whenever statistically significant heterogeneity between groups was shown by the MANOVA. The results are displayed as means and standard deviations (SD). Differences with a *P* value of 0.05 or less were considered statistically significant. Stepwise linear regression analysis

was used to estimate the contribution of changes in the concentration of plasma tHcy. SPSS for Windows version 10.0 was used for statistical analyses.

Results

Out of 45 men recruited, 43 completed the long-term study; and of these, 35 participated in the short-term study. One subject dropped out during the 2-week washout period due to abstinence symptoms from coffee drinking, and one was excluded due to dizziness during the process of drawing the blood samples. The dropped subjects were not replaced.

Baseline characteristics differed between study groups (Table 1). Age, BMI, and the activities of ALAT and γ-GT enzymes were significantly higher in the 6 cup group at study baseline when compared with 0 cup and 3 cup groups. However, the difference in the activity of ALAT in the 6 cup group was due to a high concentration in two persons (149 and 140 U/L) and the activities were not different between the groups at baseline if these subjects were excluded from the analyzes (*P* = 0.237). During the run-in period, 19 men reported withdrawal symptoms (no specific symptoms were studied), but no adverse effects because of coffee consumption during the supplementation period were reported or detected in the safety measurements (ALAT, ASAT, or γ-GT). According to two 4-day food recordings, the intake of nutrients did not differ between the study groups during the study.

The consumption of coffee increased the concentration of coffee phenols and their metabolites in urine (Table 2). Average total excretion of phenolic acids was 123 (0 cups), 109 (3 cups), and 101 (6 cups) μmol/day at baseline and 126, 152, and 157 μmol/day after the 3-week ingestion of coffee, respectively. The increases in the concentrations of

Table 2
Urinary excretion of coffee phenols and metabolites at baseline and change after 3-week supplementation period^a

	Daily coffee intake						<i>P</i> (MANOVA) ^b
	0 cups (<i>n</i> = 15)		3 cups (<i>n</i> = 14)		6 cups (<i>n</i> = 14)		
	Baseline	Change	Baseline	Change	Baseline	Change	
Caffeic acid (μmol/day)	4.1 ± 2.4	0.0 ± 1.8	3.3 ± 2.0	14.4 ± 9.2	2.5 ± 1.1	23.1 ± 8.7	<0.001
Ferulic acid (μmol/day)	24.8 ± 8.0	-1.8 ± 9.7	24.7 ± 13.9	20.2 ± 18.7	22.8 ± 12.8	41.5 ± 21.3	<0.001
<i>p</i> -Coumaric acid (μmol/day)	2.7 ± 3.7	-0.7 ± 3.6	2.8 ± 3.3	-0.4 ± 2.3	1.8 ± 1.5	-0.1 ± 1.5	0.474
Protocatechuic acid (μmol/day)	6.1 ± 4.2	-0.1 ± 4.2	4.4 ± 1.7 (13)	5.4 ± 3.7 (13)	4.2 ± 2.4 (13)	9.7 ± 3.2 (13)	<0.001
3,4-Dihydroxyphenylacetic acid (μmol/day)	25.7 ± 4.9	0.1 ± 4.3	22.8 ± 7.5 (12)	12.3 ± 15.3 (12)	21.9 ± 7.4 (9)	7.1 ± 6.8 (9)	0.012
<i>m</i> -Hydroxyphenylacetic acid (μmol/day)	59.8 ± 28.4	5.3 ± 33.1	51.7 ± 33.1	-3.8 ± 38.3	48.4 ± 23.0	-16.4 ± 17.0	0.061

^a Mean ± SD; number of subjects except where otherwise indicated in parentheses.

^b In the MANOVA age, BMI at baseline and the baseline value of the urinary concentration of phenolic acid tested were used as covariates. *P* for differences in the changes between the groups.

caffeic, ferulic, protocatechuic, and 3,4 dihydroxyphenylacetic acids were significantly different between the study groups ($P < 0.001$ for the caffeic, ferulic, and protocatechuic acids and $P < 0.05$ for the 3,4-dihydroxyphenylacetate acids). The change in the total excretion of phenolic acids in 3 and 6 cups groups represented only 3.8 and 2.5% of the daily-ingested amounts of phenolic acids.

In both coffee groups the average increase in the caffeic and ferulic acid excretion corresponded well to the increase in the average excretion of the total phenolic acids. In the 3 and 6 cup groups, on average 29 and 21% of the daily ingested ferulic acid were excreted into urine. The amount of *m*-hydroxyphenylacetic acid decreased in the supplementation groups, but increased among those not consuming any coffee. The amount of 3,4-dihydroxyphenylacetic acid increased in both coffee groups. Two

subjects in the 3 cup group and five subjects in the 6 cup group excreted after 3 weeks some unknown analyte, which coeluted with 3,4-dihydroxyphenylacetic acid and therefore it was not possible to quantify that metabolite from all the subjects.

The consumption of filtered coffee did not have long-term effects on serum lipids or short-or long-term effects on lipid peroxidation or on the activity of antioxidant enzymes (Tables 3 and 4). Plasma tHcy concentration increased nonsignificantly by 5, 16, and 26% in the 0, 3, and 6 cup groups, respectively ($P = 0.102$) (Fig. 1). However, because the mean age and BMI were different between study groups at the study baseline, we adjusted the change in the plasma tHcy for age, BMI, and the baseline concentration of tHcy in the MANOVA. After adjustment the trend in the difference of plasma tHcy change between study groups disappeared

Table 3

Concentrations of serum lipids, oxidation products of lipids, activity of antioxidant enzymes, and plasma total homocysteine (tHcy) at baseline and change after 3-week supplementation period^a

	Daily coffee intake						<i>P</i> (MANOVA) ^b
	0 cups (<i>n</i> = 15)		3 cups (<i>n</i> = 14)		6 cups (<i>n</i> = 14)		
	Baseline	Change	Baseline	Change	Baseline	Change	
Serum LDL cholesterol (mmol/L)	2.2 ± 0.8	−0.0 ± 0.3	2.3 ± 0.6	−0.0 ± 0.4	2.6 ± 0.5	−0.0 ± 0.5	0.948
Serum HDL cholesterol (mmol/L)	1.0 ± 0.1	−0.0 ± 0.1	1.1 ± 0.2	0.0 ± 0.1	1.1 ± 0.2	0.0 ± 0.1	0.324
Serum triacylglycerol (mmol/L)	1.1 ± 0.7	−0.0 ± 0.6	1.1 ± 0.6	−0.1 ± 0.3	1.3 ± 0.6	0.1 ± 0.7	0.496
Serum lipid peroxidation resistance (lagtime, min)	210 ± 23.0	−13.7 ± 28.8	199.6 ± 42.7	6.1 ± 45.8	210.4 ± 53.6	−14.6 ± 51.6	0.602
Serum LDL-conjugated dienes (μmol/mmol chol)	14.8 ± 3.6	1.3 ± 3.8	16.5 ± 4.5	−0.3 ± 4.3	16.0 ± 2.7	−0.8 ± 3.8	0.597
Plasma F ₂ -isoprostanes (pg/mL)	32.0 ± 7.3	−0.1 ± 4.9	30.1 ± 3.0	1.7 ± 7.4	31.6 ± 9.4	−0.8 ± 7.5	0.853
Plasma hydroxy fatty acids (μmol/L)	0.70 ± 0.18	−0.01 ± 0.25	0.77 ± 0.18	−0.04 ± 0.21	0.75 ± 0.17	0.09 ± 0.18	0.374
Plasma GPX (U/L) ^c	830.4 ± 134.2	23.3 ± 83.0	920.4 ± 124.6	20.4 ± 88.1	862.5 ± 115.6	61.4 ± 109.1	0.664
Serum PON (U/L) ^c	105.3 ± 63.2	−1.0 ± 7.2	114.5 ± 82.3	−0.6 ± 7.9	114.3 ± 82.6 (12)	2.4 ± 13.0 (12)	0.542
Plasma folate (nmol/L)	6.7 ± 2.1 (14)	−0.4 ± 1.3 (14)	7.8 ± 2.2 (14)	0.1 ± 3.9 (14)	6.5 ± 1.6 (13)	−0.4 ± 0.8 (13)	0.593
Plasma vitamin B ₁₂ (pmol/L)	368.9 ± 242.9	28.6 ± 210.8	436.9 ± 154.5	29.1 ± 116.2	369.3 ± 88.1 (13)	−21.1 ± 75.2 (13)	0.955
Plasma tHcy (μmol/L) ^c	11.4 ± 9.4	0.5 ± 1.2	8.6 ± 2.2	1.4 ± 1.9	11.7 ± 3.5 (12)	3.0 ± 5.0 (12)	0.494

^a Mean ± SD; Number of subjects except where otherwise indicated in parentheses.

^b In the MANOVA age, BMI at baseline and the baseline value of the parameter tested were used as a covariate. *P* for differences in the changes between the groups.

^c GPX, glutathione peroxidase; PON, paraoxonase; tHcy, total homocysteine.

Table 4
Oxidation products of lipids before and 1.5 h after consumption of coffee^a

	Daily coffee intake						<i>P</i> (MANOVA) ^b
	0 cups (<i>n</i> = 10)		1 cup (<i>n</i> = 12)		2 cups (<i>n</i> = 13)		
	Baseline	Change	Baseline	Change	Baseline	Change	
Serum LDL-conjugated dienes (μmol/mmol chol)	16.2 ± 4.8	−1.1 ± 3.7	16.2 ± 5.0	−1.4 ± 3.8 (11)	15.2 ± 2.9	−0.4 ± 1.5	0.630
Plasma hydroxy fatty acids (μmol/L)	0.69 ± 0.19	0.01 ± 0.18	0.73 ± 0.28	0.07 ± 0.23	0.84 ± 0.25	0.07 ± 0.25	0.075
Plasma F ₂ -isoprostanes (pg/mL)	31.9 ± 9.0	0.9 ± 5.0	31.8 ± 8.1	1.5 ± 6.1	30.8 ± 9.4	2.5 ± 2.5	0.853

^a Mean ± SD; number of subjects except where otherwise indicated in parentheses.

^b In the MANOVA age, BMI at baseline and the baseline value of the parameter tested were used as covariates. *P* for differences in the changes between the groups.

(*P* = 0.494). The consumption of coffee did not have an effect on plasma concentrations of folate or B₁₂ vitamin.

Even though the changes in the tHcy concentration were not significantly different between the study groups, we analyzed the contribution of the different variables on the increase in the plasma tHcy concentrations with a linear regression model. The strongest associations with the change, selected by stepwise analysis (*P* in 0.010, *P* out 0.15), were the change in urinary caffeic acid (standardized coefficient 0.65, *P* < 0.001), urinary 3,4-dihydroxyphenylacetic acid (−0.39, *P* = 0.013), and the change in serum PON activity (0.29, *P* = 0.081) (adjusted *R*² for the model 0.38, *P* = 0.001).

We also analyzed the correlation coefficient between the changes in the plasma tHcy concentration and urine concentrations of phenolic acids. The simple correlation coefficient for the association between changes in plasma tHcy and urinary caffeic acid was 0.44 (*P* = 0.004), between plasma tHcy and urinary ferulic acid 0.39 (*P* = 0.012) and between changes in plasma tHcy and urinary *p*-coumaric acid 0.30 (*P* = 0.060). Other correlations were weak and nonsignificant.

Discussion

Coffee is the most consumed beverage in the world and thus its effects on health may be highly relevant for public health. Despite extensive research the role of coffee in human health remains equivocal [1]. The aim of the present study was to test the excretion of coffee phenols and the short- and long-term effects of coffee consumption on lipid peroxidation, activity of antioxidant enzymes, and plasma tHcy concentration in healthy men.

We found that the consumption of filtered coffee increased the 24-h urinary excretion of caffeic, ferulic, protocatechuic, and 3,4-dihydroxyphenylacetic acids. The major changes of excretion were observed for caffeic and ferulic acids, but no chlorogenic acid was detected from the urine, even though it represents 90% of the coffee phenols. In general, coffee consumption affected the excretion of the phenolic compounds only modestly as the consumption of 6 cups of coffee (~700 mg of phenolic compounds) per day led only to a 55% increase in the excretion of total phenolics.

This was despite the fact that during the study the intake of coffee (other than used in the study), tea, red wine, chocolate, and cocoa was forbidden and only moderate consumption (<300 mL/day) of fruit- and berry-derived juices was allowed.

In previous studies both chlorogenic and caffeic acids have been shown to be absorbed relatively effectively (33 and 95%, respectively) in the small intestine of ileostomy subjects and caffeic acid has also been found in human plasma (peak at 1 h) and 11% of the caffeic acid has been found in urine [8,9]. However, only traces of chlorogenic acid have been detected in human plasma and urine. This is because chlorogenic acid is effectively hydrolyzed by colonic microflora into quinic acid and caffeic acid and further decarboxylated to benzoic acid [23–25]. The quinic acid moiety is further dehydroxylated into cyclohexane carboxylic acid and then aromatized into benzoic acid by microflora and excreted as hippuric acid. Hippuric acid can be formed also from caffeic acid.

In our study the consumption of coffee did not have any detectable short- or long-term effect on different measurements of lipid peroxidation or on the activity of antioxidant enzymes in healthy men. The amount of coffee used in our short-term study was 150 or 300 mL and in the long-term study 450 or 900 mL per day and

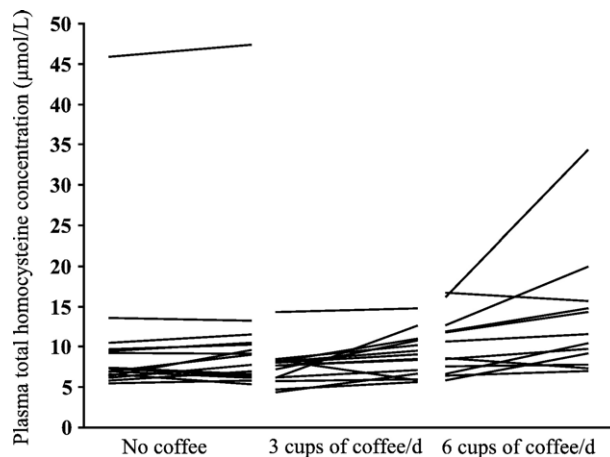


Fig. 1. The effects of long-term coffee supplementation on plasma total homocysteine.

thus delivered 120–700 mg of phenolic acids. We assume that this amount should have been sufficient to induce a change in the lipid peroxidation, if any clinically important effect would exist.

We measured lipid peroxidation using a wide variety of methods: the susceptibility of whole serum lipids to oxidation, concentration of serum LDL baseline-conjugated dienes (in vivo), plasma hydroxy fatty acids, and plasma F₂-isoprostanes. The last three measurements are in vivo markers of lipid peroxidation and of these, plasma F₂-isoprostanes are often considered to be the most reliable marker of lipid peroxidation in humans [19,26,27]. The short-term study concerned the effects on lipid peroxidation after 3 weeks of coffee supplementation and we did not measure the susceptibility of whole serum lipids to oxidation or the activity of antioxidant enzymes (GPX and PON). Therefore it is possible that some of the short-term effects could have remained undetected.

Previous data on the effects of coffee consumption on lipid peroxidation are very limited. The results of a recent study suggest that coffee might increase the antioxidant potential for a few hours in humans [10]. In that study the ingestion of 200 mL of coffee increased the antioxidant capacity by 6 and 7% at 1 h when measured as a crocin test and total radical antioxidant potential (TRAP), respectively. We did not use measurements of antioxidant capacity in our study and it is possible that coffee consumption could have had detectable effects in these measurements at least in the short-term study.

The effects of coffee consumption on the in vivo markers of lipid peroxidation have, at least to our knowledge, not been studied previously. The effects of other phenolic-rich foodstuffs, such as tea, on lipid peroxidation have, however, been studied more extensively. A substantial number of supplementation studies have demonstrated that tea increases antioxidant capacity for ~1 h, but the effects on oxidative damage are inconsistent [28]. Studies which have included in vivo markers of oxidative stress such as F₂-isoprostanes have not found any effect [29,30]. Coffee contains similar or slightly higher total amounts of phenolic compounds as tea (coffee, 70–350 mg/cup; and tea, 12–160 mg/cup), even though coffee is rich in simple phenols such as chlorogenic and caffeic, whereas tea is rich in polyphenolic compounds such as catechins and teaflavins [5,6,31]. It is possible that phenolic compounds in coffee may possess similar short-term antioxidant capacity raising effects as tea.

In our study tHcy concentrations increased by 5–26% but these changes were not significantly different between the study groups. The mean changes were mainly results of large increases in tHcy concentration in few participants as can be seen from Fig. 1. Therefore our results do not provide any unequivocal evidence that coffee would increase tHcy concentrations, but an increase in the tHcy concentration in plasma cannot be ruled out.

In previous studies, the consumption of 450–1000 mL of coffee has increased the plasma tHcy concentration by 19% at 4 h and 11–22% after 2 to 4 weeks [12,13], while in a recent short-term study consumption of coffee did not increase the concentration of tHcy [10]. It has been previously found that chlorogenic acid and caffeine are mostly responsible for the increase in tHcy, but the mechanism behind the increase is not known [11,12]. The tHcy increasing effect of caffeine was not known at the time when we conducted this study (February 2002) and coffee groups, but not the 0 cup group, were given caffeine in order to ensure compliance. This may have affected the baseline concentrations of tHcy, even though the baseline values were not different between the study groups (Table 3). According to previous studies and confirmed by our study, coffee consumption does not have an effect on the concentrations of folate, B₆ or B₁₂ vitamins [11–13].

Elevated plasma tHcy concentrations have been associated with an increased risk of cardiovascular diseases in many case-control studies and in some, but not all prospective cohort studies [15]. Even though coffee consumption would increase the tHcy concentration, there is no convincing evidence that coffee consumption would increase the risk of CVD [1]. The role of tHcy in CVD remains controversial and the relevance of the increase in the concentration of tHcy needs to be studied further.

A weakness of the current study was that the study was not randomized and double-blinded. Self-selection of study groups was used because people who normally consume coffee daily were not willing to discontinue the use and vice versa, those who do not habitually consume coffee were not willing to drink daily as high an amount as 6 cups of coffee. Selection of study group according to the previous coffee drinking habits resulted in differences in the baseline characteristics as the men in the 6 cup group were older and their BMI as well as ALAT and γ -GT concentrations were higher (Table 1). This may have had an effect on our results, even though we included age, BMI, and the baseline values of the particular variable tested as covariates in our statistical models. There were, however, no differences between the study groups in the baseline levels of oxidation markers or tHcy, and we do think that we should have detected the effect in those parameters if a true effect had existed.

We conclude that in healthy nonsmoking men, consumption of fair amounts of filtered coffee does not have any detectable short- or long-term effects on lipid peroxidation or on the activity of the main antioxidative enzymes. The effect of coffee consumption on the concentration of plasma tHcy needs further investigation.

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References

- [1] Thelle, D. S. Coffee, tea and coronary heart disease. *Curr. Opin. Lipidol.* **6**:25–27; 1995.
- [2] Steinberg, D.; Parthasarathy, S.; Carew, T. E.; Witztum, J. L. Modifications of low-density lipoprotein that increase its atherogenicity. *N. Engl. J. Med.* **320**:915–924; 1989.
- [3] Salonen, J. T.; Ylä-Herttuala, S.; Yamamoto, R.; Butler, S.; Korpela, H.; Salonen, R.; Nyyssönen, K.; Palinski, W.; Witztum, J. L. Autoantibody against oxidised LDL and progression of carotid atherosclerosis. *Lancet* **339**:883–887; 1992.
- [4] Salonen, J. T.; Nyyssönen, K.; Salonen, R.; Porkkala-Sarataho, E.; Tuomainen, T. P.; Diczfalusy, U.; Björkhem, I. Lipoprotein oxidation and progression of carotid atherosclerosis. *Circulation* **95**:840–845; 1997.
- [5] Scalbert, A.; Williamson, G. Dietary intake and bioavailability of polyphenols. *J. Nutr.* **130**:2073–2085; 2000.
- [6] Mazur, W. M.; Wähälä, K.; Rasku, S.; Salakka, A.; Hase, T.; Adlercreutz, H. Lignan and isoflavonoid concentrations in tea and coffee. *Br. J. Nutr.* **79**:37–45; 1998.
- [7] Lee, C. Antioxidant ability of caffeine and its metabolites based on the study of oxygen radical absorbing capacity and inhibition of LDL peroxidation. *Clin. Chim. Acta* **295**:141–154; 2000.
- [8] Olthof, M. R.; Hollman, P. C.; Katan, M. B. Chlorogenic acid and caffeic acid are absorbed in humans. *J. Nutr.* **131**:66–71; 2001.
- [9] Nardini, M.; Cirillo, E.; Natella, F.; Scaccini, C. Absorption of phenolic acids in humans after coffee consumption. *J. Agric. Food Chem.* **50**:5735–5741; 2002.
- [10] Natella, F.; Nardini, M.; Giannetti, I.; Dattilo, C.; Scaccini, C. Coffee drinking influences plasma antioxidant capacity in humans. *J. Agric. Food Chem.* **50**:6211–6216; 2002.
- [11] Olthof, M. R.; Hollman, P. C.; Zock, P. L.; Katan, M. B. Consumption of high doses of chlorogenic acid, present in coffee, or of black tea increases plasma total homocysteine concentrations in humans. *Am. J. Clin. Nutr.* **73**:532–538; 2001.
- [12] Verhoef, P.; Pasman, W. J.; Van Vliet, T.; Urgert, R.; Katan, M. B. Contribution of caffeine to the homocysteine-raising effect of coffee: a randomized controlled trial in humans. *Am. J. Clin. Nutr.* **76**:1244–1248; 2002.
- [13] Urgert, R.; van Vliet, T.; Zock, P. L.; Katan, M. B. Heavy coffee consumption and plasma homocysteine: a randomized controlled trial in healthy volunteers. *Am. J. Clin. Nutr.* **72**:1107–1110; 2000.
- [14] Voutilainen, S.; Alfthan, G.; Nyyssönen, K.; Salonen, R.; Salonen, J. T. Association between elevated plasma total homocysteine and increased common carotid artery wall thickness. *Ann. Med.* **30**:300–306; 1998.
- [15] Wald, D. S.; Law, M.; Morris, J. K. Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis. *BMJ* **325**:1202–1206; 2002.
- [16] Porkkala-Sarataho, E. K.; Nyyssönen, K. M.; Kaikkonen, J. E.; Poulsen, H. E.; Hayn, E. M.; Salonen, R. M.; Salonen, J. T. A randomized, single-blind, placebo controlled trial of the effects of 200 mg α -tocopherol on the oxidation resistance of atherogenic lipoproteins. *Am. J. Clin. Nutr.* **68**:1034–1041; 1998.
- [17] Ahotupa, M.; Marniemi, J.; Lehtimäki, T.; Talvinen, K.; Raitakari, O. T.; Vasankari, T.; Viikari, J.; Luoma, J.; Ylä-Herttuala, S. Baseline diene conjugation in LDL lipids as a direct measure of in vivo LDL oxidation. *Clin. Biochem.* **31**:257–261; 1998.
- [18] Wilson, R.; Smith, R.; Wilson, P.; Shepherd, M. J.; Riemersma, R. A. Quantitative gas chromatography-mass spectrometry isomer-specific measurement of hydroxy fatty acids in biological samples and food as a marker of lipid peroxidation. *Anal. Biochem.* **248** 76–85; 1997.
- [19] Morrow, J. D.; Roberts, L. J. Mass spectrometry of prostanoids: F₂-isoprostanes produced by non-cyclooxygenase free radical-catalyzed mechanism. *Methods Enzymol.* **233**:163–174; 1994.
- [20] Mackness, M. I.; Harty, D.; Bhatnagar, D.; Winocour, P. H.; Arrol, S.; Ishola, M.; Durrington, P. N. Serum paraoxonase activity in familial hypercholesterolaemia and insulin-dependent diabetes mellitus. *Atherosclerosis* **86**:193–199; 1991.
- [21] Schwab, U.; Törrönen, A.; Toppinen, L.; Alfthan, G.; Saarinen, M.; Aro, A.; Uusitupa, M. Betaine supplementation with a low calorie diet decreases plasma homocysteine concentrations but does not affect body weight, body composition, resting energy expenditure or serum lipid concentrations. *Am. J. Clin. Nutr.* **76**:961–967; 2002.
- [22] Shaw, L. M.; Stromme, J. H.; London, J. L.; Theodorsen, L. International Federation of Clinical Chemistry. Scientific Committee, Analytical Section. Expert Panel on Enzymes. IFCC methods for measurement of enzymes. Part 4. IFCC methods for gamma-glutamyltransferase [(gamma-glutamyl)-peptide: amino acid gamma-glutamyltransferase, EC 2.3.2.2]. IFCC Document, Stage 2, Draft 2, 1983-01 with a view to an IFCC Recommendation. *J. Clin. Chem. Clin. Biochem.* **10**:633–646; 1983.
- [23] Choudhury, R.; Srail, S. K.; Debnam, E.; Rice-Evans, C. A. Urinary excretion of hydroxycinnamates and flavonoids after oral and intravenous administration. *Free Radic. Biol. Med.* **27** 278–286; 1999.
- [24] Olthof, M. R.; Hollman, P. C.; Buijsman, M. N.; van Amelsvoort, J. M.; Katan, M. B. Chlorogenic acid, quercetin-3-rutinoside and black tea phenols are extensively metabolized in humans. *J. Nutr.* **133**:1806–1814; 2003.
- [25] Gonthier, M. P.; Verny, M. A.; Besson, C.; Remesy, C.; Scalbert, A. Chlorogenic acid bioavailability largely depends on its metabolism by the gut microflora in rats. *J. Nutr.* **133**:1853–1859; 2003.
- [26] Morrow, J. D.; Frei, B.; Longmire, A. W.; Gaziano, J. M.; Lynch, S. M.; Shyr, Y.; Strauss, W. E.; Oates, J. A.; Roberts, L. J., II. Increase in circulating products of lipid peroxidation (F₂-isoprostanes) in smokers. *N. Engl. J. Med.* **332**:1198–1203; 1995.
- [27] Salonen, J. T. Markers of oxidative damage and antioxidant protection: assessment of LDL oxidation. *Free Radic. Res.* **33**:41–46; 2000.
- [28] Rietveld, A.; Wiseman, S. Antioxidant effect of tea: Evidence from human clinical trials. *J. Nutr.* **133**:3285–3292; 2003.
- [29] O'Reilly, J. D.; Mallet, A. I.; McAnlis, G. T.; Young, I. S.; Halliwell, B.; Sanders, T. A.; Wiseman, H. Consumption of flavonoids in onions and black tea: lack of effect on F₂-isoprostanes and autoantibodies to oxidized LDL in healthy humans. *Am. J. Clin. Nutr.* **73**:1040–1044; 2001.
- [30] Hodgson, J. M.; Croft, K. D.; Mori, T. A.; Burke, V.; Beilin, L. J.; Puddey, I. B. Regular ingestion of tea does not inhibit in vivo lipid peroxidation in humans. *J. Nutr.* **131**:55–58; 2002.
- [31] Manach, C.; Scalbert, A.; Morand, C.; Remesy, C.; Jimenez, L. Polyphenols: food sources and bioavailability. *Am. J. Clin. Nutr.* **79**:727–747; 2004.