

# Identity of the cholesterol-raising factor from boiled coffee and its effects on liver function enzymes

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**Abstract** Boiled coffee contains an unidentified lipid that raises serum cholesterol. We studied the effects of the ingestion of coffee oil fractions of increasing purity in volunteers in order to identify the cholesterol-raising factor. In 15 volunteers who ingested 0.75 g/d of a non-triglyceride fraction from coffee oil for 4 weeks, mean cholesterol increased by 48 mg/dl (1.2 mmol/l) relative to placebo. In contrast, a coffee oil stripped of the non-triglyceride lipids cafestol and kahweol had no effect. In three volunteers, purified cafestol (73 mg/d) plus kahweol (58 mg/d) increased cholesterol by 66 mg/dl (1.7 mmol/l) after 6 weeks. Oil from Robusta beans, which contains cafestol but negligible kahweol, also raised serum cholesterol. These findings show that cafestol is at least partly responsible for the cholesterol-raising effect of boiled coffee. Coffee oils and brews containing cafestol consistently increased serum triglycerides and alanine aminotransferase, and depressed serum creatinine and  $\gamma$ -glutamyltransferase (GGT). After withdrawal GGT activity rose above baseline. Norwegians who habitually consumed 5–9 cups of boiled coffee per day had higher serum cholesterol levels and lower GGT but no higher alanine aminotransferase activity than controls. **■** Thus, serum cholesterol is raised by cafestol and possibly also kahweol, both natural components of coffee beans. The mechanism of action is unknown but is accompanied by alterations in liver function enzymes.—Weusten-Van der Wouw, M. P. M. E., M. B. Katan, R. Viani, A. C. Huggett, R. Liardon, P. G. Lund-Larsen, D. S. Thelle, I. Ahola, A. Aro, S. Meyboom, and A. C. Beynen. Identity of the cholesterol-raising factor from boiled coffee and its effects on liver function enzymes. *J. Lipid Res.* 1994. 35: 721–733.

**Supplementary key words** lipoproteins • alanine aminotransferase • randomized controlled trials • cafestol • kahweol

The consumption of Scandinavian-style “boiled coffee,” i.e., coffee prepared by boiling ground coffee beans with water and decanting the fluid into a cup without filtration,

is associated with elevated levels of serum cholesterol (1–5). In Norway, where coffee is often consumed as boiled coffee, coffee consumption is associated with an increased risk of coronary heart disease (6). In Finland, 40% of the decline in serum cholesterol over the last 25 years has been attributed to the switch from boiled to filtered coffee (7), leading to a 7% reduction in cardiovascular disease (8).

Controlled experiments have proven a cause-and-effect relationship between intake of boiled coffee and raised serum cholesterol levels (9–13), whereas drip filtered coffee has little (14) or no (3, 10–12, 15–17) effect. Boiled coffee that had been filtered before consumption had no effect on serum cholesterol levels (18, 19), indicating that the cholesterol-raising factor was retained by the paper filter. Boiled coffee contains a small amount of lipid, which is removed upon filtering (18). Such coffee lipid, when given at a daily dose of 1.3 g, corresponding to about seven cups of boiled coffee, potently elevated serum lipid levels in healthy volunteers (20). About 90% of these coffee lipids consist of fatty acids, mainly in the form of triglycerides (21). As such small amounts of fatty acids do not increase serum cholesterol (22) we proposed that the cholesterol-raising activity of coffee lipid is due to one or

Abbreviations: ALT, alanine aminotransferase; GGT,  $\gamma$ -glutamyltransferase; AST, aspartate aminotransferase; LDL, low density lipoprotein; HDL, high density lipoprotein; VLDL, very low density lipoprotein. Throughout this paper, “cafestol” and “kahweol” refer to the fatty acid esters of these compounds, but all amounts are expressed in terms of the free alcohols.

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more of its non-triglyceride, non-saponifiable components (20) which are comprised of a mixture of diterpene alcohols, sterols, hydrocarbons, squalene, and other, unknown, compounds (23).

In order to identify the cholesterol-raising compound(s) in boiled coffee we have now assessed the effect of coffee lipid fractions of increasing purity on serum lipids. In the course of our experiments we observed that coffee lipids also produced alterations in serum levels of alanine aminotransferase (ALT or SGPT) and  $\gamma$ -glutamyl-transferase (GGT). These findings prompted us to examine whether chronic long-term consumption of boiled coffee also affects serum liver enzymes.

We here report the identification of a cholesterol-raising factor in coffee and its short- and long-term effects on liver function enzymes.

## METHODS

Three original controlled studies (studies 1-3) are described below in order of increasing purity of the coffee lipid factor in the preparations used. Baseline characteristics of the subjects participating in the studies are provided in **Table 1**. As a result of novel findings concerning

effects of coffee lipids on serum liver function enzymes, serum samples from three previously reported studies were re-examined (studies 4-6). Study designs, durations, and dosages of coffee lipids are summarized in **Table 2**.

### Study 1: Non-triglyceride fraction from coffee oil

In this randomized double-blind study we tested whether the cholesterol-raising factor co-extracts with the non-triglyceride lipids from coffee oil. Coffee oil (2.5 kg) extruded from a blend of Arabica and Robusta beans (88:12, w/w) was extracted 8 times with 5 l food-grade ethanol. The extracts, which contained most of the non-triglyceride lipids, were pooled, concentrated to dryness, and redissolved in 25 l ethanol at 60°C. After cooling to 4°C, the supernatant was decanted from the precipitated triglycerides and evaporated to dryness yielding 0.52 kg of a fraction enriched fourfold in non-triglyceride components relative to the parent oil. This fraction was provided to subjects at one-fourth of the dose of coffee oil, mixed with 3 volumes of placebo oil (sunflower oil-palm oil 3:2, w/w) to maintain blinding. The triglyceride precipitate was combined with the original residue to yield the fraction depleted in non-triglyceride lipids.

The protocol and aim of the study were fully explained to the subjects, who gave their written informed consent.

TABLE 1. Designs of the studies, numbers of subjects, and amounts and types of coffee or coffee lipids ingested by subjects

Study No.	Design	Blinding <sup>a</sup>	Test Period	Number of Subjects	Coffee Type or Fraction	Intake Variables		
						Amount per Day	Cafestol <sup>b</sup>	Kahweol <sup>b</sup>
			<i>weeks</i>				<i>mg/day</i>	
1	Parallel; 2-wk run-in on placebo	Double-blind	4	16	Placebo oil <sup>c</sup>	3 g	0	0
			4	16	Coffee oil	3 g	85 <sup>d</sup>	103
			4	16	Fraction depleted in non-triglycerides	3 g	22 <sup>e</sup>	26
			4	15	Fraction enriched in non-triglycerides	0.75 g	81	98
2	Parallel; 1-wk run-in on placebo	Double-blind	4	15	Placebo oil <sup>c</sup>	2 g	0	0
			4	12	Coffee oil	2 g	57 <sup>f</sup>	69
			4	16	Coffee oil without kahweol and cafestol	2 g	0	0.3
3	Control-Test-Control	Lab-blinded	6	3	Cafestol- and kahweol-palmitate	0.13 g	73	58
4	Cross-over	Staff-blinded	4	20	Boiled coffee	8.3 dl <sup>h</sup>	17 <sup>i</sup>	14 <sup>i</sup>
					Boiled- and-filtered coffee	8.3 dl <sup>h</sup>	3 <sup>i</sup>	2 <sup>i</sup>
5	Cross-over; 2-wk wash-out	Double-blind	3	6	Arabica oil	2 g	72	53
			3	5	Robusta oil	2 g	40 <sup>e</sup>	2
6	Observational	Staff-blinded	Habitual	159	Filtered coffee	10 dl <sup>h</sup>	0.7 <sup>i</sup>	0.6 <sup>i</sup>
			Habitual	150	Boiled coffee	10 dl <sup>h</sup>	27-73 <sup>i</sup>	22-60 <sup>i</sup>

<sup>a</sup>Double: participants, investigators, and laboratory technicians were blinded to the nature of the treatments and/or the sera being analyzed. Staff: investigators and technicians were blinded. Lab: technicians were blinded.

<sup>b</sup>Expressed as mg of free nonesterified alcohol.

<sup>c</sup>Mixture of sunflower and palm oils (3:2, w/w).

<sup>d,e,f,g</sup>Plus 16-O-methylcafestol; <sup>d</sup>1 mg/day; <sup>e</sup>1.4 mg/day; <sup>f</sup>0.7 mg/day; <sup>g</sup>20 mg/day.

<sup>h</sup>Mean intake (10 dl equals 34 fluid ounces).

<sup>i</sup>Estimated from the oil content (19) of the brews and the composition of Arabica coffee oil (27).

TABLE 2. Characteristics of the participants in studies 1–3 and 6<sup>a,b</sup>

Characteristic	Study 1	Study 2	Study 3	Study 6
Sex (M/F)	31/32	20/23	3/0	167/142
Age (years)	22.2 ± 2.8	21.8 ± 1.9	49.3 ± 7.6	41 ± 1
BMI (kg/m <sup>2</sup> )	21.9 ± 1.8	21.4 ± 2.0	24.4 ± 1.7	25.0 ± 3.4
Pill users	15	11	0	4
Smokers	11	3	0	176
Serum lipids (mg/dl)				
Total cholesterol	175 ± 21	177 ± 26	197 ± 20	219 ± 41 <sup>d</sup>
LDL cholesterol	106 ± 22	104 ± 20	130 ± 9	
HDL cholesterol	53 ± 18	56 ± 12	48 ± 12	
Triglycerides	77 ± 25	81 ± 31	93 ± 23	170 ± 110 <sup>d</sup>

<sup>a</sup>Plus-minus values are means ± SD.

<sup>b</sup>Baseline characteristics for study 4 have been published (19). Those for study 5 were highly similar to those for study 1, and will be published elsewhere (Mensink, R. P., W. J. Lebbink, I. E. Lobbezoo, M. P. M. E. Weusten-van der Wouw, P. L. Zock, and M. B. Katan, in preparation).

<sup>c</sup>To convert values for cholesterol to mmol per liter, divide by 38.67. To convert values for triglycerides to mmol per liter, divide by 88.54.

<sup>d</sup>Blood lipid values refer only to the 159 subjects consuming filtered coffee. Subjects were not fasting.

Approval for the study had been obtained from the Human Ethics Committee of the Department of Human Nutrition, Wageningen Agricultural University. At this time we were unaware of the effects of coffee oil on serum ALT levels. During a run-in period of 2 weeks, all 63 participants received 8 capsules daily, containing a total of 3 g placebo oil. Subjects swallowed 4 capsules in the morning and 4 in the evening. During the next 4 weeks, subjects received either placebo oil, coffee oil, coffee oil depleted in non-triglyceride lipids, or non-triglyceride-enriched coffee oil. No treatment was given in the 4-week follow-up period. Other details are given in Table 1. Recruitment and selection of subjects were similar to previous studies (24); baseline characteristics are given in Table 2. The participants were instructed to maintain their usual dietary and living habits, not to consume more than six cups of filtered coffee per day, and to abstain from non-filtered coffee. Diaries kept by subjects showed that they had taken 20,974 out of the 21,168 capsules provided. One woman dropped out at the start of the test period because she disliked the taste of the capsules. Food intake was checked in three 24-h recalls (25); group mean changes in serum cholesterol as predicted from changes in fat and cholesterol intake (22, 26) ranged from –3 to –0.2 mg/dl and were thus of no consequence.

#### Study 2: Coffee oil stripped of cafestol and kahweol

The major components of the non-triglyceride fraction of coffee oil are palmitate and linoleate esters of the diterpenoid alcohols, kahweol and cafestol (27). A coffee oil, prepared by hexane extraction of spent coffee grounds, was depleted of kahweol and cafestol by counter-current steam-stripping at 215°C at a pressure of 1 mm Hg. Residual cafestol and kahweol was less than 1% of that originally present (Table 1). After a run-in period of 1 week on placebo oil, groups of 12–16 subjects took 2 g (5 capsules) per day of the stripped oil, untreated coffee

oil, or placebo oil for 4 weeks, in a double-blind parallel design (Table 1). Other conditions were the same as for study 3. Two 24-h dietary recalls indicated that changes in serum cholesterol due to diet changes were less than 5 mg/dl (22, 26). The volunteers had taken 8,005 out of 8,050 capsules provided.

Two subjects withdrew from the study due to reasons unrelated to treatment. In three subjects treatment was switched to a lower dose by an independent safety monitor, because their serum cholesterol had increased by more than 60 mg/dl. One of them was later found to have had a baseline serum ALT level of 215 U per l (upper limit of normal, 54 U per l) and a GGT of 550 U per l (upper limit of normal for men, 99 U per l). His data were excluded, as were those of the other two subjects. Inclusion of the values of the latter two resulted in only marginal changes in the results, e.g., an increase in serum ALT in the coffee oil group of 31 U per l (95% CI 13 to 48 U per l,  $P < 0.01$ ) instead of 35 U per l (95% CI 17 to 53,  $P < 0.01$ ).

#### Study 3: Pure cafestol- plus kahweol-palmitate

The results of study 2 were compatible with the hypothesis that cafestol and/or kahweol are the cholesterol-raising factors. We therefore tested the effect of a mixture of highly purified cafestol- and kahweol-palmitate in three particularly well-informed volunteers (including authors MBK and RV) who came forward of their own accord. One was located in Wageningen, one in Nijmegen, and one in Vevey, Switzerland. Since we were now aware of the influence of coffee oil on serum ALT, the protocol, which was approved by the Committee on Experimental Research in Man of the Nijmegen University Hospital, included the expected effects on serum ALT.

Cafestol plus kahweol (55:45, w/w) were isolated from coffee oil by transesterification in alkaline methanol (28), and the free alcohols were re-esterified with palmitoyl

chloride. The amount of purified sample sufficed for only three volunteers. The final product contained 92% by weight of cafestol- and kahweol-monopalmitates, and 8% dipalmitate esters, free cafestol, free kahweol, and free palmitic acid. Separation of the two diterpenoids proved not to be feasible. The esters were dissolved in placebo oil (sunflower oil-palm oil, 3:2) to a concentration equivalent to that in coffee oil (Table 2).

The subjects consumed 2 g (5 capsules) of placebo oil daily during the run-in period of 3 weeks, 2 g of placebo oil containing kahweol and cafestol during the test period of 6 weeks, and placebo oil for a 5 week follow-up period. At days 17, 21, and 24 of the test period, one subject was not available for blood sampling.

#### Study 4: Boiled versus boiled-and-filtered coffee

Serum samples from a published 2 × 4 week cross-over study (19), which had been stored for 24 months at -70°C, were analyzed for liver function enzyme activities to determine whether short-term consumption of boiled coffee affects serum liver enzymes, and whether the coffee component responsible is retained by a paper filter. The 20 volunteers had received 5 to 9 cups (mean 0.83 l or 28 fluid ounces) of coffee per day (19).

#### Study 5: Robusta versus Arabica coffee oil

ALT levels were analyzed in serum samples from a double-blind 2 × 3 week cross-over study with a 2-week wash-out period (Mensink R. P., W. J. Lebbink, I. E. Lobbezoo, M. P. M. E. Weusten-van der Wouw, P. L. Zock, and M. B. Katan, unpublished results) to check whether the serum ALT-raising factor is present in oil from both *Coffea arabica L.* and *Coffea robusta L.* beans. Three subjects had received Arabica oil during period 1, two had received Robusta oil, and six a placebo consisting of a 1:1 mixture of palm and sunflower oils. After a 2-week wash-out period those subjects who had received placebo in period 1 received Arabica (n = 3) or Robusta (n = 3) oil for 3 weeks, and those who had received coffee oil received placebo. The sera had been stored at -80°C for 32 months.

#### Study 6: Cross-sectional study in Norwegians

In order to determine whether the effects observed in studies 1-5 were chronic or transient, we analyzed liver function enzymes in non-fasting serum samples of 142 Norwegian women and 167 men reporting to consume five or more cups per day of either boiled (n = 150) or filtered coffee (n = 159) (cf. Tables 1 and 2). The study was designed to detect a difference in serum ALT activity of 4 U per l between the two groups with a power of 90% and  $P = 0.05$ . Samples were collected as part of the Norwegian National Health screening (4). The participants were all healthy, and they were not on any chronic medication known to affect liver enzymes. They also reported

that they did not consume more than three alcoholic beverages per day.

#### Blood sampling and chemical analyses

Fasting sera, coded to maintain blinding, were stored at -80°C. All samples obtained from the same subject were analyzed within one run. Sera were analyzed enzymatically for total (29) and HDL cholesterol (29, 30) and triglycerides (31, 32). Mean bias for control sera provided by the Centers for Disease Control (Atlanta, GA) was -2% for total cholesterol, -0.4% for HDL, and 4% for triglycerides (studies 1-3 and 5). The coefficient of variation within runs ranged from 0.7% to 2.1%. LDL cholesterol was calculated (33). In study 6, lipids were assayed as previously described (4). Creatinine was measured according to a modified Jaffé method (34) using a kit from Boehringer (Mannheim, Germany). Creatine kinase activity was also determined using a Boehringer kit (35). Bilirubin was measured according to Jendrassik and Gróf (36). For these three variables, mean bias with regard to the target values provided by the Dutch Foundation for Quality Control of Hospital Laboratories ranged from -1.2% to 4%. For studies 3-6, serum activities of ALT (37), aspartate aminotransferase (AST or SGOT) (37), GGT (38, 39), and alkaline phosphatase (40) were measured at 37°C using Abbott Spectrum reagents (Abbott Laboratories, North Chicago, IL). The mean bias for 'Monitrol' control sera (Baxter Dade, Switzerland) ranged from -0.3% to 1%. The coefficient of variation within runs ranged from 0.8% to 10%. In studies 1 and 2, enzyme activities were determined at 30°C using reagents from Boehringer, Mannheim, Germany (ALT (41), AST (42), GGT (39, 43), and alkaline phosphatase (44)). Mean bias with regard to the target values provided by the Dutch Foundation for Quality Control of Hospital Laboratories ranged from -11% to -5%. Enzyme activities measured in split serum samples at the two temperatures showed linear relationships, with correlation coefficients  $r \geq 0.95$  and slopes ( $\pm$  SEM) of 2.29 ( $\pm$  0.03) for ALT, 1.56 ( $\pm$  0.02) for AST, 1.54 ( $\pm$  0.08) for GGT, and 1.43 ( $\pm$  0.02) for alkaline phosphatase. All enzyme activities obtained at 30°C were recalculated to the equivalent values at 37°C.

For analysis of cafestol and kahweol, samples were hydrolyzed with ethanolic KOH, 2 mol/l, after addition of 5- $\alpha$  cholestane and betulin as internal standards. The free diterpene alcohols were extracted with diethylether, trimethylsilylated with hexamethyldisilazane-trichloromethylsilane 2:1 (v/v), and separated on a 25 m × 0.22 mm fused silica CP Sil5CB column (Chrompack, Middelburg, The Netherlands) using hydrogen as carrier gas. The authenticity and purity of peaks was verified by mass spectrometry on a Finnegan model 8430 mass spectrometer coupled to a Hewlett-Packard 5890 gas chromatograph and using pure compounds as standards.

## Statistical analysis

For studies 1 and 2, the mean of the two values obtained at the end of the run-in placebo period was subtracted from the mean at the end of the test period to yield each subject's response. Mean responses were compared among treatment groups with the General Linear Models procedure of the Statistical Analysis System (45). When the analyses indicated a significant effect of treatment ( $P < 0.05$ ), a *t*-test was used to compare treatments. **Table 3** provides the differences in response between the various treatment groups and the placebo group. In study 3, the mean of the three values obtained during the run-in period was subtracted from the mean of the final three values obtained during the test period and the difference was compared against zero using a paired Student's *t*-test. Studies 4 and 5 were originally designed as cross-over trials. However, there were obvious carry-over effects of treatment in the first period into the second treatment period. Therefore, only period 1 was used for the statistical analyses. Responses were calculated as the change from baseline during period one. The mean response on boiled coffee was then compared with that on boiled-and-filtered coffee (study 4) and the responses on Arabic and

Robusta oil with that on placebo oil (study 5) using an unpaired *t*-test. In study 6, the two groups were compared using an unpaired Student's *t*-test.

## RESULTS

### Study 1: Non-triglyceride fraction from coffee oil

A coffee oil fraction enriched in non-triglyceride lipids (0.75 g/day) produced increases in serum cholesterol and triglycerides similar to those produced by 3 g/day coffee oil (**Fig. 1**, Table 3). Serum cholesterol increased with time, and after 4 weeks it had not yet reached a plateau. The oil fraction depleted in non-triglyceride lipids had significantly less effect on serum lipids than the enriched fraction ( $P = 0.02$  for cholesterol,  $P = 0.00$  for triglycerides). On average, 78% of the rise in total cholesterol produced by the treatments was due to LDL. HDL cholesterol declined on coffee oil.

Concomitant effects were also seen on serum ALT and GGT activities. ALT was raised by coffee oil and by the oil fraction enriched in non-triglyceride lipids; a significantly smaller rise ( $P = 0.01$ ) was seen with the

TABLE 3. Responses of serum lipids, liver function enzymes, and creatinine to consumption of coffee oil enriched or depleted in nonsaponifiable lipids (study 1), to coffee oil stripped of cafestol and kahweol (study 2), and to pure cafestol plus kahweol (study 3)

Study No.	Treatment	Cholesterol <sup>a</sup>	Triglycerides <sup>a</sup>	High Density Lipoprotein <sup>a</sup>	ALT	Creatinine	GGT	
							Treatment Period <sup>b</sup>	Post-Experiment <sup>c</sup>
			mg/dl		U/l	μmol/l	U/l	
1	Coffee oil	49	73	-5	41	-6	-3	13
	95% CI	(37,61)	(51,95)	(-8,-1)	(20,61)	(-9,-3)	(-5,-1)	(4,22)
	<i>P</i>	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01
	Enriched fraction	48	66	-1	47	-7	-4	14
	95% CI	(36,59)	(44,88)	(-5,3)	(26,68)	(-10,-4)	(-5,-2)	(5,23)
	<i>P</i>	<0.01	<0.01	0.57	<0.01	<0.01	<0.01	<0.01
	Depleted fraction	33	29	1	18	-3	-2	3
	95% CI	(22,45)	(8,51)	(-2,5)	(-3,38)	(-6,0)	(-4,-0)	(-6,12)
	<i>P</i>	<0.01	<0.01	0.45	0.09	0.04	0.02	0.49
2	Coffee oil	49	73	-2	35	-5	-4	9
	95% CI	(34,64)	(56,89)	(-6,3)	(17,53)	(-8,-2)	(-6,-1)	(2,16)
	<i>P</i>	0.01	<0.01	0.50	<0.01	<0.01	<0.01	0.02
	Stripped oil	4	-0	2	-4	2	-1	0
	95% CI	(-10,18)	(-16,15)	(-3,6)	(-20,13)	(-0,5)	(-3,1)	(-7,7)
	<i>P</i>	0.58	0.98	0.48	0.65	0.08	0.45	0.99
3	Cafestol + kahweol	66	162	-13	31	-4	-3	13
	[Lowest;highest]	[53;89]	[102;266]	[-22;-6]	[12;56]	[-6;-2]	[-4;-2]	[8;16]
	<i>P</i>	0.03	0.09	0.10	0.13	0.11	0.04	0.03

Values for studies 1 and 2 represent the mean response in the treatment group minus the mean response in a concurrent placebo group, and the 95% confidence interval and *P* value of this difference. Values for study 3 represent mean changes from the end of the baseline to the end of the treatment period; figures in brackets are ranges.

<sup>a</sup>To convert to mmol/l, divide cholesterol by 38.67 and triglycerides by 88.54.

<sup>b</sup>GGT activity after 1 week of treatment in studies 1 and 3, and 2 weeks of treatment in study 2.

<sup>c</sup>GGT activity after cessation of treatment relative to values at the end of the treatment period.

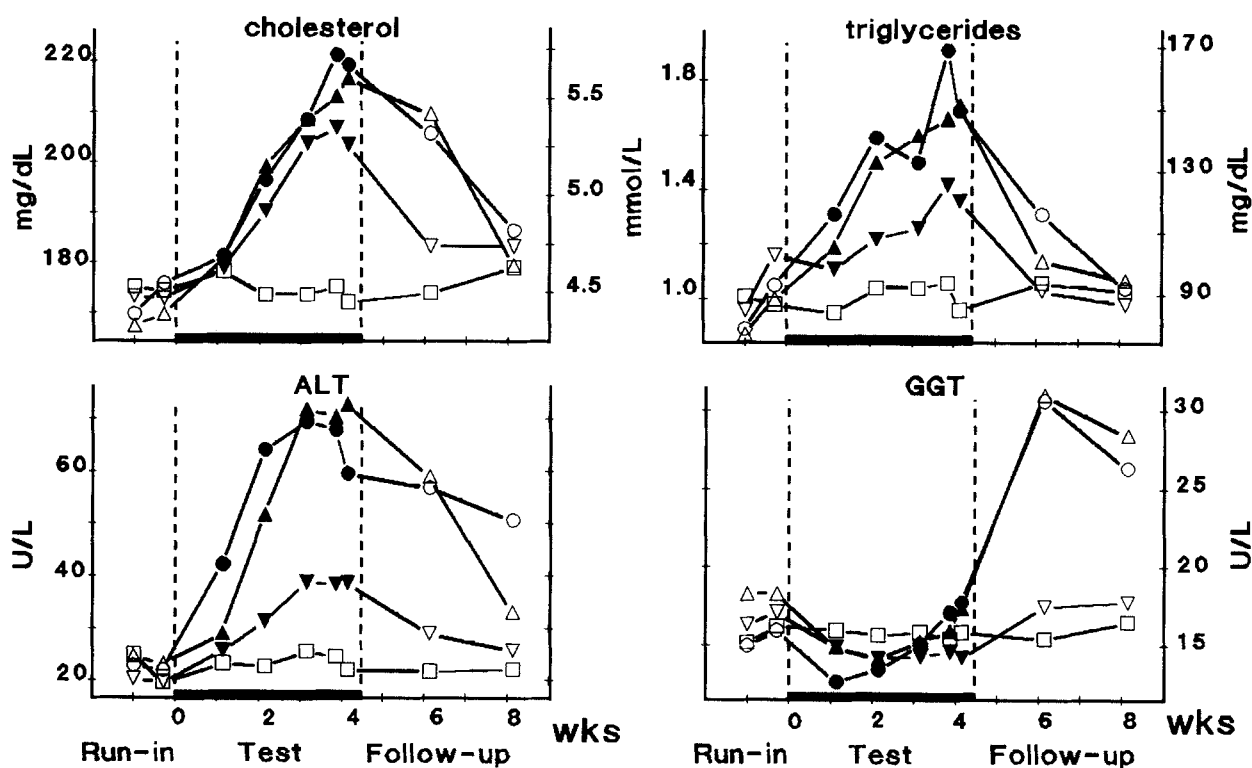


Fig. 1. Study 1: Influence of 3 g per day of placebo oil (□), coffee oil (●), of coffee oil depleted in non-triglyceride lipids (▼), or of 0.75 g per day of coffee oil enriched in non-triglyceride lipids (▲) on serum cholesterol, triglycerides, serum ALT, and GGT activity in healthy volunteers ( $n = 15$  or 16 per group). The horizontal black bar indicates the test period. All subjects received 3 g per day of placebo oil during the run-in period and no treatment during the follow-up period.

depleted oil fraction. The three treatments lowered GGT activity after 1 week, which in the groups given coffee oil or the enriched fraction was followed by a rebound rise that peaked at 2 weeks after withdrawal of treatment. Enzyme levels had returned to baseline in 47 subjects checked 1 year later. Serum creatinine concentrations were lowered by the three treatments, more so by the enriched than by the depleted fraction ( $P = 0.01$ ). Serum alkaline phosphatase, aspartate aminotransferase (AST or SGOT), creatine kinase, and bilirubin were not systematically influenced (data not shown).

### Study 2: Coffee oil stripped of cafestol and kahweol

Consumption of 2 g of coffee oil versus placebo oil produced effects on serum lipids, enzyme activities, and serum creatinine similar to those in study 1 (Fig. 2 and Table 3). In contrast, 2 g per day of coffee oil stripped of cafestol and kahweol had no effect on serum cholesterol, triglycerides, creatinine, or activities of ALT or GGT. In the placebo group mean cholesterol changed by 2 mg/dl and ALT by 4 U/l; changes in other variables were also minimal and insignificant.

Serum lactate dehydrogenase, iron, calcium, phosphate, amylase, albumin, protein, urea, and urate levels measured in five randomly chosen subjects from the coffee

oil group, seven from the placebo group and six from the stripped-oil group were unaffected by consumption of coffee oil (data not shown).

### Study 3: Pure cafestol- plus kahweol-palmitate

Purified cafestol plus kahweol significantly raised serum cholesterol levels in all three volunteers confirming that one or perhaps both of these components are responsible for the cholesterol-raising effects of boiled coffee and coffee lipids. Most (71%) of the rise was associated with LDL (Fig. 3, Table 3). The effects on cholesterol were accompanied by a rise in serum triglycerides and serum ALT activity, and a decline in HDL cholesterol and creatinine levels. GGT activity fell after 1 week of treatment, but was elevated after withdrawal of treatment. Bilirubin levels decreased, but AST activities and alkaline phosphatase levels were not affected (data not shown).

### Study 4: Boiled versus boiled-and-filtered coffee

Effects of the coffees on serum lipids have been reported previously and are summarized in Table 4. In those subjects who first consumed boiled coffee, serum ALT activity increased nonsignificantly relative to the placebo group ( $P = 0.08$ ), and then decreased when they were switched to boiled-and-filtered coffee (Fig. 4, Table 4).

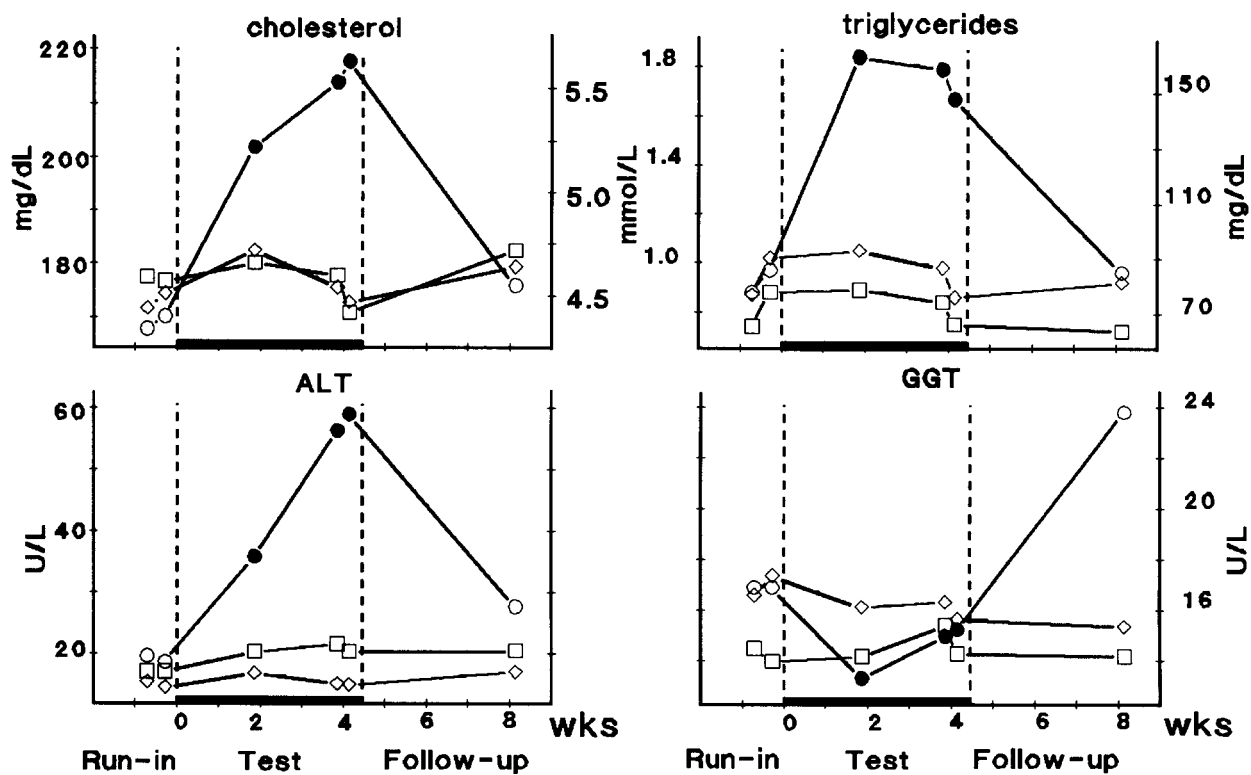


Fig. 2. Study 2: Influence of 2 g per day of placebo oil (□ and open symbols during the run-in period), coffee oil (●), or coffee oil stripped of cafestol and kahweol (◇) on serum cholesterol, triglycerides, serum ALT, and GGT activity in healthy volunteers (n = 12–16 per group). The test period is indicated by a horizontal black bar. Subjects received 2 g per day of placebo oil during the run-in period and no treatment during the follow-up period.

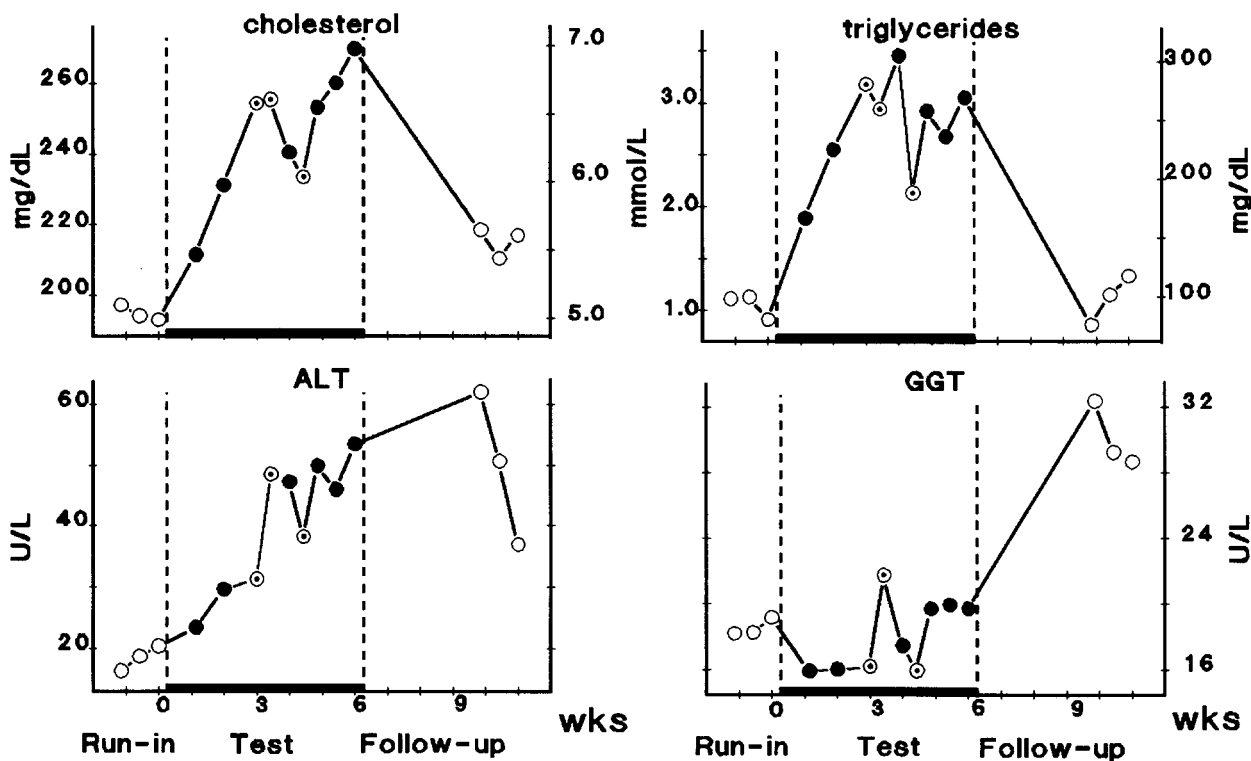


Fig. 3. Study 3: Influence of 73 mg cafestol plus 58 mg kahweol per day on serum cholesterol, triglycerides, serum ALT, and GGT activities in three healthy volunteers (●). The test period is indicated by a horizontal black bar. Subjects received 2 g per day of placebo oil (○) during the run-in and follow-up periods. On three days of the test period one volunteer was not available for blood sampling, therefore (⊙) indicates mean of two values only.

TABLE 4. Responses of serum lipids and liver function enzymes to consumption of boiled coffee, Arabica coffee oil, and Robusta coffee oil (studies 4 and 5), and mean differences in lipids and enzymes between subjects habitually consuming boiled coffee and subjects consuming filtered coffee (study 6)

Study No.	Treatment	Cholesterol <sup>c</sup>	Triglyceride <sup>a</sup>	High Density Lipoprotein <sup>a</sup>	ALT	GGT <sup>b</sup> (On Treatment)
			mg/dl		U/l	U/l
4	Unfiltered, boiled coffee <sup>d</sup>	8	42	-6	7	-12
	95% CI	(-13,29)	(-3,88)	(-12,-1)	(-1,-16)	(-28,4)
	<i>P</i>	0.45	0.07	0.02	0.08	0.13
5	Arabica oil	31	39	-3	14	-1
	[Lowest; highest]	[25,35]	[34;44]	[-15;11]	[5,25]	[-2;-1]
	<i>P</i>	0.02	0.14	0.56	0.01	0.30
	Robusta oil	28	40	6	9	-3
	[Lowest;highest]	[19;37]	[39;41]	[4,7]	[8;10]	[-6;-0]
	<i>P</i>	0.09	0.21	0.01	<0.01	0.16
6	Unfiltered, boiled coffee <sup>d</sup>	12	20	1	-6	
	95% CI	(3,21)	(-8,47)	(-1,4)	(-11,-2)	
	<i>P</i>	0.01	0.17	0.29	0.01	

Studies 4 and 5 were cross-over trials, but values represent the mean response during period 1 minus the mean response in a concurrent control group; period 2 was not analyzed because of carry-over. For studies 4 and 6, values in parentheses are 95% confidence intervals of the difference; for study 5 they are ranges, in brackets.

<sup>a</sup>To convert to mmol/l, divide cholesterol by 38.67 and triglyceride by 88.54.

<sup>b</sup>GGT activity relative to control group after 4 weeks of treatment in study 4, 3 weeks of treatment in study 5, and chronic consumption of boiled coffee in study 6.

<sup>c</sup>Unfiltered boiled coffee, n = 10; filtered boiled coffee, n = 8.

<sup>d</sup>Non-fasting samples.

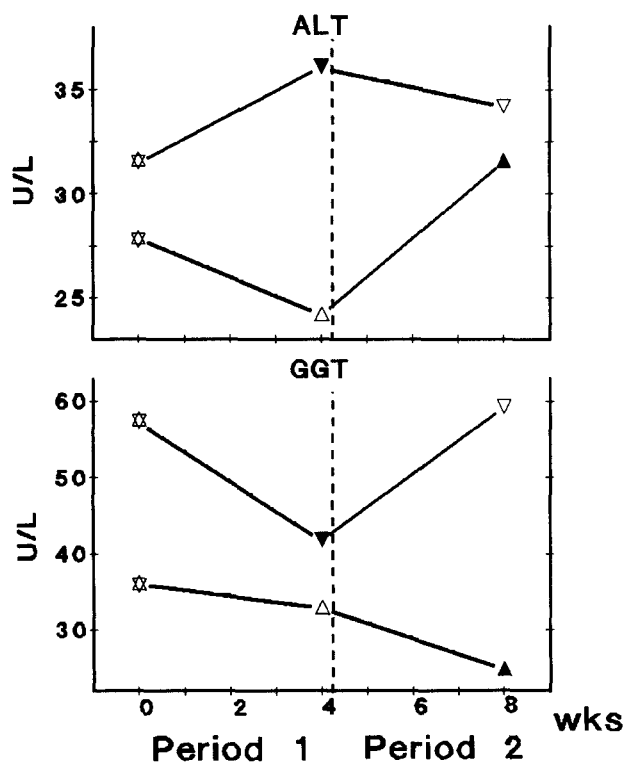


Fig. 4. Study 4: Serum ALT and GGT activity in volunteers consuming on average 7 cups/day of boiled (▲, ▼) or boiled-and-filtered coffee (△, ▽) in a cross-over study (n = 9 or 11 per group).

However, it did not return to baseline levels within 4 weeks. Those subjects who started out on boiled-and-filtered coffee in period 1 showed a rise in serum ALT activity after the switch to boiled coffee. Because of the carry-over effect (19), only the results of treatment period 1 were analyzed statistically. GGT activities were lowered nonsignificantly ( $P = 0.13$ ) on boiled coffee. Alkaline phosphatase and AST activities did not differ between treatments (data not shown).

#### Study 5: Robusta versus Arabica coffee oil

In this cross-over study we also saw a carry-over effect of treatment. In period 1 both Arabica and Robusta oil caused a rise in serum ALT activity compared with the combined placebo groups ( $P = 0.01$  and  $0.00$ , respectively). In those subjects who had consumed coffee oil during weeks 1 to 3, ALT remained above baseline levels at week 8 (Fig. 5, Table 4). Serum cholesterol was increased significantly in period 1 during consumption of Arabica ( $P = 0.02$ ) and nonsignificantly ( $P = 0.09$ ) during consumption of Robusta oil. GGT and alkaline phosphatase activity declined slightly; AST activity was not influenced (data not shown).

#### Study 6: Cross-sectional study in Norwegians

In subjects habitually consuming five or more cups of boiled coffee per day, the mean serum cholesterol level was 232 mg/dl (6.0 mmol/l), significantly higher than the



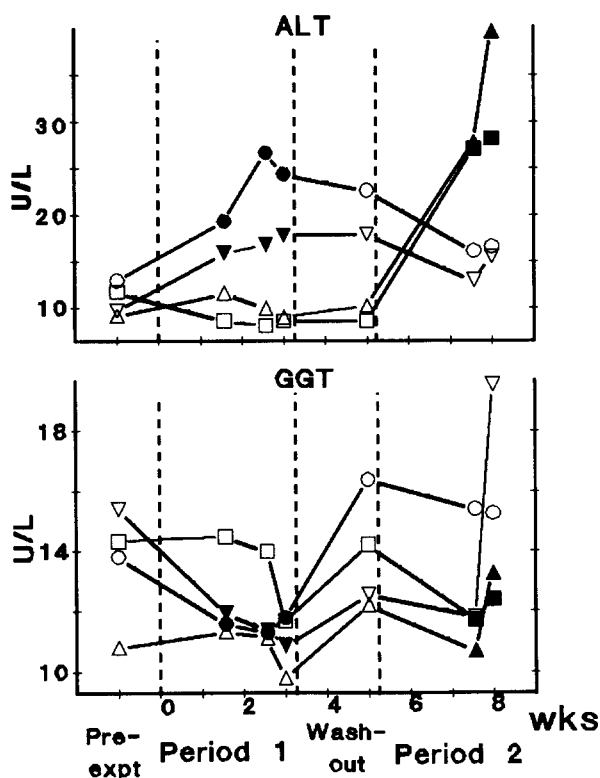


Fig. 5. Study 5: Serum levels of ALT and GGT in volunteers consuming 2 g/day of Arabica coffee oil ( $\blacktriangle$ ,  $\bullet$ ), Robusta coffee oil ( $\blacksquare$ ,  $\blacktriangledown$ ) or placebo oil ( $\triangle$ ,  $\nabla$ ,  $\square$ ,  $\circ$ ) ( $n = 2$  or  $3$  per group).

mean of 219 mg/dl (5.7 mmol/l) in the matched filter coffee drinkers, but the two groups had similar serum ALT activities (Table 4). GGT activities were significantly lower in drinkers of boiled coffee, while serum AST and alkaline phosphatase levels were not different from those in filter coffee drinkers (data not shown).

## DISCUSSION

### Identification of the cholesterol-raising factor

Our results show that cafestol, possibly together with kahweol (Fig. 6), is responsible for the cholesterol-raising effect of boiled coffee (9–13) and coffee lipids (20). We have previously shown that the cholesterol-elevating factor is associated with small traces of lipid present in boiled but not in filtered coffee (18, 19). We have now found that it is associated with the non-triglyceride portion of these lipids and furthermore we have demonstrated that consumption of a purified mixture of cafestol-palmitate and kahweol-palmitate, the most abundant constituents of the non-triglyceride lipids (27), elevated serum cholesterol levels, whereas coffee oil stripped of cafestol and kahweol had no effect. The results of study 5 suggest that both Robusta oil, which contains cafestol and 16-O-methyl-

cafestol but no kahweol (46–48), and Arabica oil, which contains cafestol and kahweol (49), elevate serum cholesterol. Taken together, these findings suggest the involvement of cafestol in raising cholesterol levels but they do not exclude an additional role for kahweol or some of the minor coffee diterpenes. The disappearance of the cholesterol-raising effect of boiled coffee after filtration through a paper filter (18, 19) can now be explained by the retention of cafestol and possibly kahweol by the filter.

In order to derive semi-quantitative estimates for the effects of coffee diterpenes on serum cholesterol and ALT activity, we combined the data of studies 1–5 into single dose-response plots. The results for kahweol and cafestol were similar; therefore only the plots for cafestol are given (Fig. 7). The comparison of the change in serum cholesterol levels with the consumption of cafestol across the five controlled trials (Fig. 7A) indeed suggests a dose-response relationship, each extra 2 mg of cafestol consumed producing a 1 mg/dl increase in cholesterol. The inconsistencies in the literature as to the effect of various types of coffee on serum cholesterol (1, 3, 10–12, 14–17) most likely relate to different cafestol plus kahweol contents of the various brews. In the cross-sectional study in Norwegians, serum cholesterol concentrations were 12 mg/dl higher in subjects habitually drinking boiled coffee than in those drinking filtered coffee. Based on the dose-response curve (Fig. 7A) their estimated cafestol intake of 27–73 mg per day (Table 2) should theoretically have led to a difference in cholesterol levels of 26–49 mg/dl. Possibly we have overestimated actual cafestol intakes, due to adherence of coffee lipids to the cup or to errors in the volume or number of cups consumed. Alternatively, habitual boiled coffee drinkers may show a partial adaptation to the effects of cafestol.

About 75% of the increase in serum cholesterol induced by coffee oil and cafestol was due to an increase of

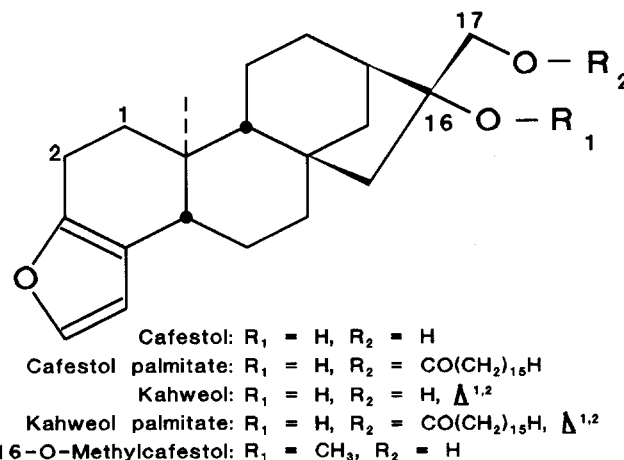


Fig. 6. Structure of the diterpenoid alcohol cafestol (23). Kahweol has an additional double bond between carbon atoms 1 and 2 (23).

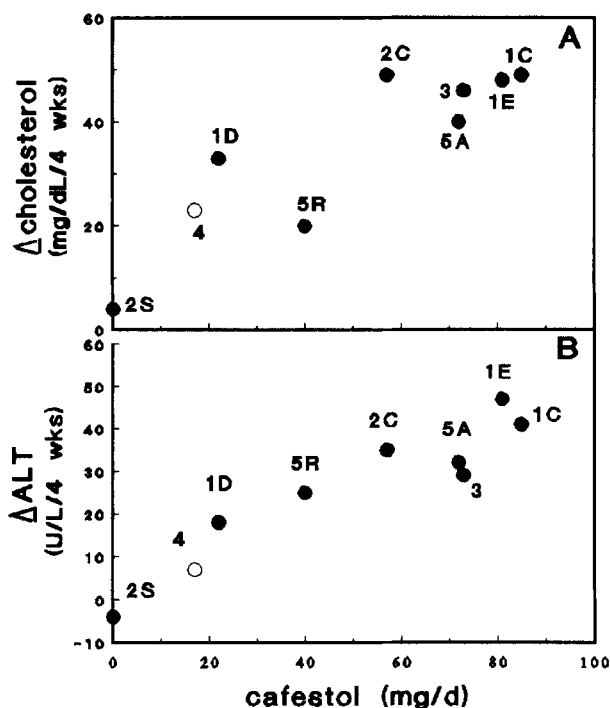


Fig. 7. Relation of average cafestol intake per group (Table 1) with observed response of serum cholesterol (A) and ALT activity (B) across the five controlled studies. The responses are given for a treatment period of 4 weeks; those found in study 2 after 3 weeks were multiplied by 1.33. For studies 1 and 2, responses are only given for the groups that first received the control treatment and then the test treatment. Codes refer to studies and treatments: 1C, intact coffee oil; 1E, fraction enriched in non-triglyceride lipids; 1D, fraction depleted in non-triglyceride lipids; 2C: intact coffee oil; 2S, oil stripped of kahweol and cafestol; 3, pure cafestol plus kahweol; 4, boiled coffee; 5A, Arabica coffee oil; 5R, Robusta coffee oil. Linear regression equations were  $\Delta$ cholesterol (mg/dl) =  $12.1 + 0.5 \times$  cafestol (mg/d) (panel A) and  $\Delta$ ALT (U/l) =  $1.1 + 0.5 \times$  cafestol (mg/d) (panel B).

LDL cholesterol. In agreement with earlier observations with boiled coffee and coffee lipids (18, 19), HDL cholesterol concentrations were slightly lowered by cafestol. A considerable proportion of the cafestol-induced rise in cholesterol was thus due to a rise in VLDL cholesterol. The effect on VLDL and triglycerides was more marked in the present studies using purified coffee lipids than in earlier studies (18–20) using boiled coffee. A possible explanation is the higher dose of cafestol in the present trials.

#### Effects on serum ALT activity

The large majority of subjects in studies 1–5 were not accustomed to drink boiled coffee. Over the 3- to 6-week period when they were exposed to cafestol, their serum ALT activity increased in a dose-related manner, each 2 mg of cafestol producing a 1 U per liter increase in ALT (Fig. 7B). Four weeks after withdrawal of treatment average serum ALT activity was still elevated, but it had returned to normal levels 1 year later in the subjects analyzed at that time.

There was only a minimal and insignificant difference in serum ALT between Norwegians habitually consuming boiled coffee compared to those drinking filtered coffee. This difference is 14–37 U per liter less than that predicted from the controlled studies (Fig. 7B and Table 1), indicating that the effect of cafestol plus kahweol on serum ALT is transient and disappears after prolonged exposure. As the groups drinking boiled coffee and filtered coffee were well matched, it is unlikely that an effect of chronic consumption of boiled coffee on serum ALT was masked by some opposing factor.

#### Mechanisms

The effects of cafestol plus kahweol on serum lipids and serum ALT did not reach a steady state within 3–6 weeks of daily administration. The reason for this delayed effect is unknown but could be due to a slow accumulation of cafestol and kahweol or their active metabolites in the liver. The elevation of serum ALT persisted longer than that of cholesterol after cafestol withdrawal, indicating that cafestol might affect serum ALT and cholesterol through distinct pathways. The mild nonpathological increase in serum ALT also implicates an effect of cafestol on the liver cell. The lack of effect on serum bilirubin and alkaline phosphatase activities appears to exclude cholestasis, and the lack of constant effects on AST argues against any pronounced alteration of hepatocyte integrity.

The decreased serum GGT activity with boiled coffee observed in the cross-sectional study in Norwegians confirms the findings of the Tromsø study (50, 51). This effect was also noted in our controlled trials, in which a rebound increase was observed after withdrawal of coffee lipids. Whether these effects are mediated through changes in synthesis of GGT by liver cells (52) or through other mechanisms is unknown, but these findings again suggest that the liver is the target organ for cafestol-mediated perturbation of cholesterol homeostasis.

We can offer no explanation for the decrease in serum creatinine levels provoked by coffee oil and cafestol. Further studies of kidney function and urinary excretion of creatinine in subjects consuming cafestol or kahweol may clarify this observation.

#### Health implications

Cafestol plus possibly kahweol, substances that naturally occur in coffee beans, have potent serum cholesterol elevating activity in humans. Their presence in other plants has not been reported. The separate levels of cafestol and kahweol in various brews of coffee are as yet unknown, but Ratnayake et al. (53) have recently shown that total lipid and diterpene contents per serving are highest in boiled coffee, the coffee type most frequently associated with increased serum cholesterol levels. Hypercholesterolemic patients in Scandinavia are advised to switch from boiled to filtered coffee (54). Middle East

("Turkish") coffee as consumed in countries such as Greece, Turkey, and Israel is also relatively high in diterpenes, but the bioavailability of cafestol in such coffee may be different because some of it is probably contained in floating coffee particles. Still, in Israel a significant positive correlation was found between coffee consumption and serum lipids (55). The effects on serum lipids of moderate consumption of the other types of brews are probably insignificant. The low serum cholesterol levels in Italy (56) and Greece (56) suggest that neither espresso nor Greek coffee have an important cholesterol-raising effect, and various studies have demonstrated that instant (57, 58) and filtered (3, 10–12, 15–17) coffees have no effect on serum cholesterol levels.

Cafestol plus possibly kahweol caused a rise in serum ALT activity during short-term trials, but not in chronic users of boiled coffee. Mortality from chronic liver disease and cirrhosis is lower in populations drinking boiled coffee such as that of Finland (6.5 per 100,000, age-standardized), Sweden (4.7), and Norway (5.0), than in the USA (9.6), France (16.7), or Italy (22) (59). This argues against any major role of boiled coffee in liver disease. In addition, in our studies with boiled coffee and coffee oils, the levels of the serum liver enzymes remained within the normal range. Thus, warnings against the consumption of unfiltered coffee in patients at risk of liver disease are not warranted.

In conclusion, cafestol is a potent cholesterol-raising factor, responsible at least in part, for the hypercholesterolemic effects of boiled coffee. Cafestol may provide a tool to increase our understanding of the regulation of cholesterol metabolism in the liver, and thus might aid in the development of new and better drugs to control dyslipoproteinemias. ■

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