

# Separate effects of the coffee diterpenes cafestol and kahweol on serum lipids and liver aminotransferases<sup>1-3</sup>

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**ABSTRACT** The coffee diterpene cafestol occurs in both robusta and arabica beans. It is present in unfiltered coffee brews and raises serum concentrations of cholesterol, triacylglycerols, and alanine aminotransferase in humans. The effects are linear with the cafestol dose. Unfiltered coffee also contains the related compound kahweol, which occurs only in the major coffee strain arabica. The activity of kahweol is unknown. In a randomized, double-blind crossover study, we gave 10 healthy male volunteers either pure cafestol (61–64 mg/d) or a mixture of cafestol (60 mg/d) and kahweol (48–54 mg/d) for 28 d. Relative to baseline values, cafestol raised mean ( $\pm$  SEM) total serum cholesterol concentrations by  $0.79 \pm 0.14$  mmol/L ( $31 \pm 5$  mg/dL), low-density-lipoprotein (LDL) cholesterol by  $0.57 \pm 0.13$  mmol/L ( $22 \pm 5$  mg/dL), fasting triacylglycerols by  $0.65 \pm 0.12$  mmol/L ( $58 \pm 11$  mg/dL), and alanine aminotransferase by  $18 \pm 2$  U/L (all  $P < 0.01$ ). Relative to cafestol alone, the mixture of cafestol plus kahweol increased total cholesterol by another  $0.23 \pm 0.16$  mmol/L ( $9 \pm 6$  mg/dL) ( $P = 0.08$ ), LDL cholesterol by  $0.23 \pm 0.16$  mmol/L ( $9 \pm 6$  mg/dL) ( $P = 0.09$ ), triacylglycerols by  $0.09 \pm 0.10$  mmol/L ( $8 \pm 9$  mg/dL) ( $P = 0.20$ ), and alanine aminotransferase by  $35 \pm 11$  U/L ( $P = 0.004$ ). Thus, the effect of cafestol on serum lipid concentrations was much larger than the additional effect of kahweol, and the hyperlipidemic potential of unfiltered coffee mainly depends on its cafestol content. Both cafestol and kahweol raised alanine aminotransferase concentrations, and their hyperlipidemic effect thus seems not to be coupled with their effect on liver cells. *Am J Clin Nutr* 1997;65:519–24.

**KEY WORDS** Alanine aminotransferase, coffee lipids, crossover trial, enzymes, humans, lipoprotein metabolism, unfiltered coffee

## INTRODUCTION

Unfiltered, boiled coffee raises serum concentrations of low-density-lipoprotein (LDL) and very-low-density-lipoprotein (VLDL) cholesterol in humans (1–7). Coffee brews prepared without a filter contain 1–2 g lipids/L, of which  $\approx 10\%$  are diterpenes (8). In a series of controlled experiments (9, 10), we showed that diterpenes are responsible for the cholesterol-raising effects of unfiltered coffee. The relation appeared to be linear up to doses of 200 mg diterpenes/d (10), the amount in 3–5 L (20–30 cups) of boiled or French-press coffee (11).

The mode of action of coffee diterpenes is largely unknown. They raised serum concentrations of alanine aminotransferase

and reduced those of  $\gamma$ -glutamyltransferase in humans (10, 12). These alterations may point to changes in the integrity of liver cells (13). In addition, coffee diterpenes reduced circulating lipoprotein(a) concentrations (Urgert et al, unpublished observations, 1996), which may also be related to changes in liver cell metabolism (14). We therefore suggest that coffee diterpenes influence lipoprotein metabolism via effects on the liver.

The major coffee diterpenes are cafestol and kahweol (Figure 1). (Throughout this paper, cafestol and kahweol refer to the fatty acid esters of these compounds, but amounts are expressed in terms of the unesterified alcohols.) Robusta beans contain mainly cafestol, whereas arabica beans also contain high amounts of kahweol. Daily ingestion of oils pressed from robusta beans increased serum lipid concentrations in humans (15, 16), indicating that cafestol has hyperlipidemic potential. However, in the United States and in Western Europe, arabica beans are preferred to robusta beans (17). As a result, amounts of kahweol in unfiltered coffee brews are higher than those of cafestol (11). However, previous studies did not allow conclusions on the activity of kahweol (15, 16).

We studied the effects of kahweol on serum concentrations of lipoproteins and liver enzymes by comparing the effects of pure cafestol with a mixture of cafestol and kahweol in a crossover trial with healthy volunteers.

## SUBJECTS AND METHODS

### Preparation of diterpenes

Because it proved impossible to prepare pure kahweol in sufficient quantities (Kosmeijer-Schuil et al, unpublished observations, 1996), we decided to compare the effects of a mixture of cafestol and kahweol with those of cafestol alone.

The purified diterpenes were prepared from coffee oil by Nestec Ltd (Vevey, Switzerland) for the first treatment period and by our own laboratory for the second treatment period.

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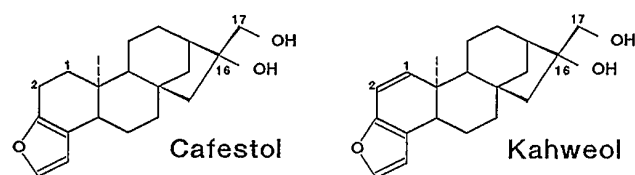


FIGURE 1. Structure of the coffee diterpene alcohols cafestol and kahweol. Compared with cafestol, kahweol has an additional double bond between the C-1 and C-2 carbon atoms. Diterpenes occur in coffee beans mainly esterified to fatty acids at the C-17 position.

Diterpenes naturally occur as fatty acid esters, mostly of palmitic acid (18). Esters were saponified with anhydrous potassium hydroxide, and the diterpene alcohols extracted with methanol-water and dichloromethane-methanol solutions. Active carbon was used to bind nonditerpene compounds. Finally, warm methanol was added and the solution cooled to allow the mixture of diterpenes to crystallize. Pure cafestol was obtained by adding gaseous hydrogen to a mixture of cafestol and kahweol with PdPbCaCO<sub>3</sub> as a catalyst. The diterpene alcohols were reesterified with palmitoylchloride in a pyridine solution. The solvent was purified over a chromatographic column charged with aluminium oxide.

The purified diterpenes were dissolved in sunflower oil plus palm oil (3:2, by wt). Red palm oil was used to mask the yellowish color of kahweol.  $\beta$ -carotene in the supplements provided < 2% of the recommended daily intake of vitamin A for Dutch adult men (19). Diterpene contents of the capsules were assayed as described (10). Purities of the diterpenes ranged from 92.2% to 99.7%; impurities consisted of free cafestol and kahweol, cafestol and kahweol dipalmitate, and palmitic acid. The pyridine content was less than the detection limit of 0.25 mg/g.

### Design and subjects

The study lasted from September 1994 to April 1995 (Figure 2). Prior approval was obtained from the Human Ethics Committee of our department and from the Nijmegen University Hospital Ethical Committee.

We estimated that ingestion of 60 mg cafestol plus a similar amount of kahweol daily would raise serum cholesterol concentrations by 1.1 mmol/L (10). If cafestol and kahweol were similarly active, each would account for a rise of 0.55 mmol/L. Calculations showed that we needed 10 subjects to detect a difference of 0.55 mmol/L with a statistical power of 90% using a crossover design ( $\alpha = 0.05$ ).

We recruited 10 volunteers by displaying posters in university buildings and through personal contact. All were male and most were students at Wageningen Agricultural University. We

carefully explained to them the study protocol and the expected changes in serum lipids and liver enzymes. Subjects then provided their written informed consent. The subjects filled out a medical questionnaire, which was reviewed by an independent internist at the Department of Gastroenterology, Nijmegen University Hospital. None of the subjects reported a history of gastrointestinal, liver, or kidney disease; none had glucosuria or proteinuria; all were considered to be in good health on examination by the internist; and none were taking medication known to affect serum lipids or liver enzymes. All test results were within normal limits, and none of the subjects had serum antibodies to hepatitis B core antigen or to hepatitis C virus. The mean ( $\pm$  SD) age was  $24 \pm 4$  y and the mean body mass index (in kg/m<sup>2</sup>) was  $21 \pm 2$ . Two of the subjects smoked.

An independent investigator grouped the subjects into pairs on the basis of similar cholesterol concentrations. Within each pair, subjects were randomly allocated to one of two treatment sequences by tossing a coin. During the run-in periods, subjects swallowed five placebo capsules providing a total of 2 g of a mixture of sunflower and palm oils (3:2, by wt) daily. During the first treatment period, five subjects swallowed five capsules per day, providing 64 mg cafestol and 1 mg kahweol (amounts expressed as free alcohols) dissolved in 2 g of placebo oil. The other five subjects swallowed capsules with 60 mg cafestol and 54 mg kahweol, again dissolved in oil. Supplements were switched in the second treatment period; analyzed doses of cafestol and kahweol were now 61 and 0 mg for pure cafestol, and 60 and 48 mg for the placebo-oil mixture, respectively. Every 2 wk the subjects were provided with new capsules, which they stored in a refrigerator. They took two capsules at breakfast and three capsules with their evening meals, and reported the time of consumption in a special diary. No supplements were given during the washout and follow-up periods. Subjects as well as investigators were blinded to the supplement sequences. The code was broken after completion of both the blood assays and the statistical analyses.

Subjects were asked to maintain their usual dietary and living habits during the run-in and treatment periods, to abstain from types of coffee other than paper-filtered or instant (soluble) coffee, and to restrict alcohol use to a maximum of 20 alcohol-containing beverages/wk. Subjects kept daily records of coffee and alcohol consumption, medication use, and any deviations from their dietary and living habits. Body weights were measured at every blood sampling in subjects without shoes and heavy clothes.

Venous blood samples were obtained after an overnight fast on days 15 (run-in), 36, 39, 43 (treatment), 57 (washout), 106 (second run-in), 127, 130, 134 (second treatment), and 148 and 183 (follow-up) (Figure 2). Additional serum samples were

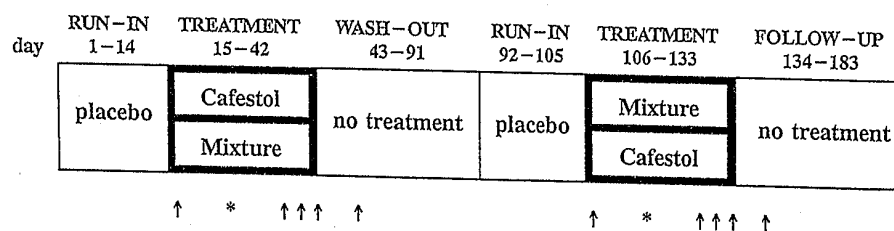


FIGURE 2. Experimental design. "Cafestol" refers to the palmitate esters of cafestol alone, and "Mixture" refers to the palmitate esters of cafestol and kahweol. Blood samples (↑) were obtained on days 15 (run-in), 36, 39, 43 (treatment), 57 (washout), 106 (run-in), 127, 130, 134 (treatment), and 148 and 183 (follow-up). Additional serum samples (\*) were obtained to monitor adverse effects during the treatment periods.

obtained on the 15th day of either treatment period, and were analyzed instantly for total cholesterol and alanine aminotransferase concentrations so as to monitor adverse effects. These values were seen by the internist and not by the investigators. Predefined safety limits were a concentration of alanine aminotransferase higher than the upper limit of normal (53.5 U/L), or a rise in total cholesterol of  $> 2.0$  mmol/L over baseline. As a result, three subjects (two in the first and one in the second treatment period) were switched to placebo-oil capsules because of raised alanine aminotransferase concentrations. All three subjects later turned out to have been taking supplements with cafestol plus kahweol. Statistical analyses were done both with and without their values.

### Blood sampling and assays

Serum was obtained by centrifugation and stored at  $-80^{\circ}\text{C}$ . Serum was analyzed enzymatically for total (20) and high-density-lipoprotein (HDL) cholesterol (21) and triacylglycerols (22). Mean bias for control serum provided by the Centers for Disease Control and Prevention (Atlanta) was  $-1\%$  for total and HDL cholesterol and  $10\%$  for triacylglycerols. The CV within runs ranged from  $0.9\%$  to  $1.7\%$ . LDL was calculated by using the Friedewald method (23). Creatinine was measured with a modified Jaffé method by using a Spectrum kit (Abbott Laboratories, North Chicago) (24). Concentrations of alanine aminotransferase and aspartate aminotransferase (25),  $\gamma$ -glutamyltransferase (26), and alkaline phosphatase (27) were measured at  $37^{\circ}\text{C}$  by using Abbott Spectrum reagents. The mean bias for these enzymes in Monitrol control serum (Baxter Dade, Düringen, Switzerland) ranged from  $-1\%$  to  $3\%$ . The within-run CV ranged from  $2\%$  to  $8\%$ . Upper limits of normal according to the manufacturer were  $53.5$  U/L for alanine aminotransferase,  $39.7$  U/L for aspartate aminotransferase,  $92$  U/L for alkaline phosphatase, and  $63$  U/L for  $\gamma$ -glutamyltransferase. All samples from one subject were analyzed in the same run, except for serum samples obtained at the end the second follow-up period (day 183), which were analyzed in a separate run.

### Statistical analyses

For each subject, treatment values were means of the three values obtained at the end of the treatment periods. The effects of either treatment were analyzed by subtracting the values obtained after the preceding run-in period from the treatment values; the effects of the mixture relative to cafestol alone were analyzed by subtracting the values after cafestol from those after the mixture.

Three subjects were switched to placebo halfway through the treatment. Their final values were estimated as follows. We calculated the mean daily change from treatment day 15 until the end of the treatment period for the seven subjects who completed the treatment period. The mean daily change was  $0.029$  mmol/L for total cholesterol,  $0.031$  mmol/L for LDL,  $-0.003$  mmol/L for HDL,  $0.003$  mmol/L for triacylglycerols,  $1.7$  U/L for alanine aminotransferase,  $0.4$  U/L for aspartate aminotransferase,  $-0.2$  U/L for alkaline phosphatase, and  $-0.1$  U/L for  $\gamma$ -glutamyltransferase. This change was multiplied by the number of days of treatment that the other three subjects had missed, and this value was added to their value obtained at the time when treatment was stopped. We also did

analyses by using their actual values at the time they were taken off treatment (eg, end values) as well as analyses without these three subjects.

All variables were normally distributed. None of the variables showed significant carryover or period effects. Differences were therefore tested against zero with one-sided paired *t* tests. We used the SAS statistical package for all analyses (28).

### RESULTS

According to the diaries kept by the subjects,  $> 99\%$  of the capsules were taken. During the treatment periods none of the subjects took any medication known to affect liver function, and the mean use of alcohol-containing beverages was  $< 1$  beverage/d. The mean ( $\pm$  SD) change in body weight was  $0.3 \pm 0.8$  kg (range:  $-0.4$  to  $2.3$  kg) over the first, and  $-0.7 \pm 0.9$  kg (range:  $-1.8$  to  $1.3$  kg) over the second treatment period.

### Serum lipids

Relative to baseline values, consumption of cafestol alone raised the mean ( $\pm$  SEM) concentration of total cholesterol by  $0.79 \pm 0.14$  mmol/L, LDL cholesterol by  $0.57 \pm 0.13$  ( $P = 0.002$ ), and fasting triacylglycerols by  $0.65 \pm 0.12$  mmol/L ( $P < 0.001$ ); it reduced HDL cholesterol by  $0.06 \pm 0.04$  mmol/L ( $P = 0.06$ ). Relative to cafestol alone, consumption of the mixture of cafestol and kahweol raised total cholesterol by  $0.23 \pm 0.16$  mmol/L ( $P = 0.08$ ), LDL cholesterol by  $0.23 \pm 0.16$  mmol/L ( $P = 0.09$ ), and triacylglycerols by  $0.09 \pm 0.10$  mmol/L ( $P = 0.20$ ) (Table 1, Figure 3).

Three subjects were switched to receive placebo halfway through treatment with the mixture of cafestol and kahweol. When we took their serum values at the time of switching as end values, the effects of the mixture relative to cafestol alone were  $0.17 \pm 0.15$  mmol/L for total cholesterol ( $P = 0.15$ ),  $0.15 \pm 0.15$  mmol/L for LDL cholesterol ( $P = 0.16$ ), and  $0.08 \pm 0.10$  mmol/L for triacylglycerols ( $P = 0.23$ ) ( $n = 10$ ).

When we excluded from the analyses the three subjects who were taken off treatment, cafestol raised total cholesterol by  $0.83 \pm 0.19$  mmol/L ( $P = 0.003$ ), LDL cholesterol by  $0.74 \pm 0.13$  mmol/L ( $P < 0.001$ ), and triacylglycerols by  $0.64 \pm 0.10$  mmol/L ( $P < 0.001$ ), relative to baseline. Relative to cafestol alone, the mixture raised total cholesterol by  $0.08 \pm 0.16$  mmol/L ( $P = 0.31$ ), LDL cholesterol by  $0.06 \pm 0.16$  mmol/L ( $P = 0.36$ ), and triacylglycerols by  $0.05 \pm 0.12$  mmol/L ( $P = 0.34$ ) ( $n = 7$ ).

### Other serum variables

Cafestol alone raised alanine aminotransferase by  $18 \pm 2$  U/L over baseline values ( $P < 0.001$ ). Intake of the mixture further raised alanine aminotransferase by  $35 \pm 11$  U/L for the full group of 10 subjects ( $P = 0.004$ ) (Table 1, Figure 3). With serum values at the time of switching as end values for the three subjects who were taken off treatment, the effect of the mixture relative to cafestol was  $31 \pm 9$  U/L ( $P = 0.004$ ). When the three subjects who were switched to placebo were excluded, alanine aminotransferase rose by  $18 \pm 2$  U/L with cafestol alone ( $P < 0.001$ ), and by another  $20 \pm 7$  U/L ( $P = 0.02$ ) with the mixture ( $n = 7$ ).

TABLE 1

Serum concentrations of lipids, lipoproteins, liver enzymes, and creatinine in 10 healthy male volunteers after consumption of 61–64 mg cafestol/d alone or of a mixture of 60 mg cafestol/d plus 48–54 mg kahweol/d for 28 d each in a crossover study<sup>1</sup>

	Cafestol			Mixture			Mixture minus cafestol	
	Pretreatment	Posttreatment	Change	Pretreatment	Posttreatment	Change	Difference (95% CI)	P
Total cholesterol (mmol/L)	4.80 ± 0.99 <sup>2</sup>	5.59 ± 1.05	0.79 ± 0.44 <sup>3</sup>	4.88 ± 0.89	5.82 ± 1.01	0.94 ± 0.61 <sup>3</sup>	0.23 (–0.05, 0.52)	0.08
HDL cholesterol (mmol/L)	1.49 ± 0.41	1.42 ± 0.39	–0.06 ± 0.13	1.48 ± 0.35	1.39 ± 0.39	–0.09 ± 0.21	–0.03 (–0.10, 0.04)	0.20
LDL cholesterol (mmol/L) <sup>4</sup>	2.96 ± 0.78	3.53 ± 0.87	0.57 ± 0.41 <sup>5</sup>	3.05 ± 0.73	3.75 ± 0.77	0.71 ± 0.49 <sup>5</sup>	0.23 (–0.06, 0.51)	0.09
Triacylglycerols (mmol/L)	0.75 ± 0.21	1.40 ± 0.38	0.65 ± 0.38 <sup>3</sup>	0.77 ± 0.27	1.48 ± 0.61	0.71 ± 0.39 <sup>3</sup>	0.09 (–0.09, 0.26)	0.20
Alanine aminotransferase (U/L)	23 ± 5	41 ± 10	18 ± 7 <sup>3</sup>	30 ± 9	76 ± 40	46 ± 37 <sup>5</sup>	35 (16, 54)	0.004
Aspartate aminotransferase (U/L)	22 ± 6	27 ± 5	5 ± 3 <sup>3</sup>	27 ± 9	40 ± 13	12 ± 15 <sup>6</sup>	12 (6, 18)	0.002
γ-Glutamyltransferase (U/L)	17 ± 6	16 ± 5	–1 ± 2 <sup>5</sup>	19 ± 7	16 ± 6	–3 ± 2 <sup>5</sup>	–0 (–1, 0)	0.19
Alkaline phosphatase (U/L)	78 ± 21	76 ± 27	–1 ± 11	77 ± 19	72 ± 23	–5 ± 9	–4 (–9, 1)	0.09
Creatinine (μmol/L)	89 ± 10	86 ± 10	–3 ± 8	90 ± 12	82 ± 9	–8 ± 5 <sup>3</sup>	–4 (–8, –0)	0.04

<sup>1</sup> Pretreatment (baseline) values were determined 2 wk after treatment with placebo oil began, on day 15, and on day 106. Posttreatment values were determined after diterpene intake and are averages of measurements on days 36, 39, and 43 in the first period and of those on days 127, 130, and 134 in the second period. To convert mmol/L to mg/dL multiply by 38.67 for cholesterol, and by 88.54 for triacylglycerols.

<sup>2</sup>  $\bar{x} \pm SD$ .

<sup>4</sup> Calculated according to Friedewald et al (23).

<sup>3,5,6</sup> Significantly different from zero: <sup>3</sup>  $P < 0.001$ , <sup>5</sup>  $P < 0.01$ , <sup>6</sup>  $P < 0.05$ .

Aspartate aminotransferase increased less than alanine aminotransferase, whereas γ-glutamyltransferase decreased after both cafestol and the mixture. Two weeks after cafestol treatment ended, γ-glutamyltransferase had risen by  $7 \pm 2$  U/L over baseline values, and 2 wk after treatment with the mixture ended, γ-glutamyltransferase had risen by  $4 \pm 2$  U/L over baseline values (both  $P < 0.05$ ). Alkaline phosphatase and creatinine in serum tended to be lowered both by cafestol alone and by the mixture of cafestol and kahweol (Table 1). Seven weeks after withdrawal of the supplements, all serum variables had returned to baseline values (data not shown).

## DISCUSSION

Intake of cafestol raised the serum concentrations of total and LDL cholesterol and those of fasting triacylglycerols in healthy male volunteers. The supplement providing a mixture of cafestol and kahweol had little additional effect on serum lipids. This finding suggests that kahweol has less capacity than cafestol to interfere with lipid metabolism in humans. Cafestol and kahweol both raised aminotransferase concentrations in serum, which suggests that they both affect liver cells.

Three subjects were switched to placebo halfway through the cafestol and kahweol treatment because their serum concentration of alanine aminotransferase had exceeded the upper limit of normal. Their final values were recalculated by imputation from the remaining seven subjects. However, statistical analyses based on only the seven subjects who completed the full treatment, as well as analyses in which the values obtained at the time of switching to placebo were taken as end values for the three subjects who were taken off treatment, led to the same conclusions: cafestol was the main lipid-raising factor from coffee beans, and both cafestol and kahweol raised alanine aminotransferase.

The amount of coffee diterpenes we supplied per day was equivalent to that in 10–20 cups of boiled Turkish or French-press coffee (11). We chose a high dose so as to reach the necessary power with the limited amount of purified diterpenes available. Lower doses of diterpenes applied to a larger population would have decreased the power of our study, because

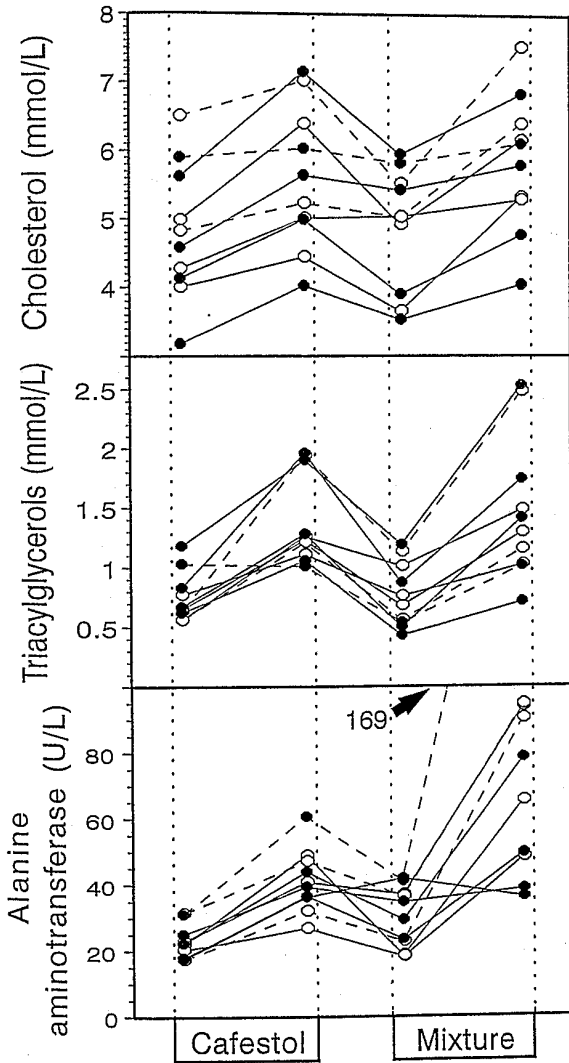
previous studies showed that the responses would be smaller but the SDs of the responses would not (10). Because women tend to respond less to coffee diterpenes than do men (Urgert et al, unpublished observations, 1996), we included only male volunteers in the study to further minimize possible variation in responses.

Although we could separate cafestol and kahweol on an analytical scale (11), semipreparative HPLC gave very low yields for purified diterpenes, especially of kahweol. We therefore decided to compare supplements of cafestol alone with a mixture of cafestol and kahweol.

## Effects of cafestol and kahweol on serum lipids

Consumption of pure cafestol increased serum total cholesterol by 17%, LDL cholesterol by 19%, and fasting triacylglycerols by 86%, whereas consumption of a mixture of cafestol and kahweol further increased total cholesterol by only 2%, LDL cholesterol by 4%, and triacylglycerols by 7%. The effect of coffee diterpenes on lipid cholesterol follows a dose-response relation, which appears to be linear up to a dose of 200 mg cafestol plus kahweol/d (10). Thus, if cafestol and kahweol were similarly active, the effect of the mixture on cholesterol concentrations should have been about twice that of the pure cafestol. Kahweol thus appears to be less hyperlipidemic than cafestol.

The separate effects of coffee diterpenes were examined earlier indirectly in studies comparing the effects of robusta oil, which contains mainly cafestol, and arabica oil, which contains cafestol and kahweol (Figure 4). Van Rooij et al (15) found that robusta oil increased serum cholesterol by 11%, which is consistent with our finding that cafestol is hypercholesterolemic. The supplement of arabica oil in their study contained five times as much cafestol plus kahweol as the supplement of robusta oil, largely because of higher amounts of kahweol. However, the effect of arabica oil on serum cholesterol was only twice that of robusta oil. Mensink et al (16) found increases of 13% with both arabica and robusta oil, even though the intake of cafestol plus kahweol was three times higher with arabica oil than with robusta oil. These results are consistent

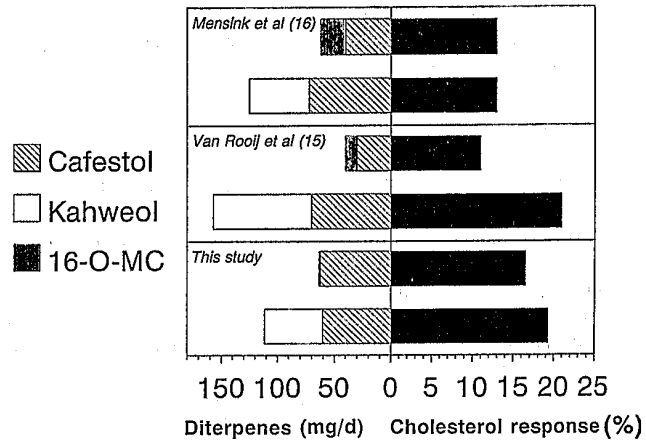


**FIGURE 3.** Individual baseline values and changes in serum concentrations of cholesterol, triacylglycerols, and alanine aminotransferase after ingestion of either 61–64 mg cafestol/d alone (cafestol) or of a mixture of 60 mg cafestol/d plus 48–54 mg kahweol/d (mixture) for 28 d each by 10 healthy male volunteers in a crossover study. ●: Subjects who first received cafestol and then the mixture; ○: subjects who received the opposite sequence. The three subjects who were switched to placebo halfway through the mixture treatment are indicated by broken lines.

with our finding that kahweol has little additional effect on the serum concentration of cholesterol.

The mechanisms by which coffee diterpenes affect cholesterol synthesis or breakdown in the human body are largely unknown. Kahweol only differs from cafestol in that it has a double bond between C-1 and C-2 (Figure 1). Possibly, this double bond allows faster biotransformation into compounds that are rapidly excreted or that have less hyperlipidemic capacity.

It is not known whether 16-*O*-methylcafestol—a diterpene present in robusta but not in arabica beans (29)—also affects serum lipoproteins. However, 16-*O*-methylcafestol accounts for only 3% of the diterpenes present in commercial roast and ground coffees (11), and intakes are therefore low.



**FIGURE 4.** Comparison of present results with results from studies that examined the effects of coffee oils containing mixtures of cafestol, kahweol, and 16-*O*-methylcafestol (16-*O*-MC) on cholesterol responses. Mensink et al (16) gave five volunteers placebo or robusta oil (upper bar), and six placebo or arabica oil (lower bar) for 3 wk each in a crossover design. Van Rooij et al (15) gave placebo, robusta (upper bar), or arabica oil (lower bar) to three groups of 12 subjects for 6 wk in a parallel design. We gave 10 volunteers pure cafestol (upper bar) or a mixture of cafestol plus kahweol (lower bar) for 4 wk each in a crossover design.

**Effects of cafestol and kahweol on serum liver enzymes**

Intake of cafestol alone raised the mean concentrations of liver aminotransferases; the addition of kahweol strongly increased the responses. This indicates that the C-1–C-2 double bond of kahweol does not reduce its hepatocellular effect. Alanine aminotransferase rose more than did aspartate aminotransferase, which is consistent with results from previous studies (10, 12, 15). In the liver, alanine aminotransferase occurs mainly in the cytoplasm, whereas aspartate aminotransferase is predominantly localized in mitochondria (13). Possibly, coffee diterpenes disturb the permeability of cell membranes in the liver parenchyma, but do not cause the extensive damage needed to release enzymes from mitochondria.

Concentrations of  $\gamma$ -glutamyltransferase were reduced on intake of coffee diterpenes and showed a rebound increase of  $\approx$ 30% after withdrawal of both supplements. A similar pattern was observed in previous studies (10). The serum concentration of  $\gamma$ -glutamyltransferase is largely determined by the liver, where  $\gamma$ -glutamyltransferase occurs predominantly in epithelial cells of the biliary duct (13). The discrepant effects of cafestol and kahweol on  $\gamma$ -glutamyltransferase and alanine aminotransferase suggest that coffee diterpenes (or their metabolites) may have various sites of action in the liver.

**Conclusion**

Consumption of unfiltered coffee brews elevates serum lipoprotein concentrations mainly through their cafestol content, whereas kahweol has little additional effect. Both diterpenes elevated serum liver aminotransferases, indicating that lipid metabolism and liver function may be affected by coffee diterpenes through different pathways. Elucidation of the underlying mechanisms may produce new insights into lipoprotein metabolism.

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