Effects of cafestol and kahweol from coffee grounds on serum lipids and serum liver enzymes in humans[1–3]

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ABSTRACT The diterpenes cafestol and kahweol are present in unfiltered coffee in oil droplets and floating fines. They elevate serum cholesterol and alanine aminotransferase (ALT). We measured fines in coffee brews, and examined diterpene availability from spent grounds in healthy volunteers. Turkish or Scandinavian boiled coffee contained 2–5 g fines/L and French press coffee contained 1.5 g fines/L. An intake of 8 g fine grounds/d for 3 wk increased cholesterol by 0.65 mmol/L (95% CI 0.41–0.89 mmol/L) and ALT by 18 U/L (95% CI 4–32 U/L) relative to control subjects (n = 7/group). In a crossover study (n = 15), mean serum cholesterol was 4.9 mmol/L after consumption of both fine and coarse grounds for 10 d (P = 0.43). Serum ALT activities were 29 U/L on fine and 21 U/L on coarse grounds (P = 0.02). Floating fines could contribute substantially to the hyperlipidemic and ALT-elevating effect of unfiltered coffee. Diterpene measurements in coffee brews should include the contribution of fines. *Am J Clin Nutr* 1995;61:149–54

KEY WORDS Cafestol, kahweol, coffee grounds, serum cholesterol, alanine aminotransferase, humans

Introduction

Scandinavian-type boiled coffee, prepared by boiling coarsely ground coffee beans with water and decanting the fluid without filtration, elevates serum cholesterol and triglycerides (1–4). The diterpenoid alcohol cafestol, possibly together with kahweol, is responsible for this effect (5). (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.) Cafestol plus kahweol esters also elevate at least transiently the serum activity of alanine aminotransferase (ALT) in serum and depress serum concentrations of creatinine in humans not accustomed to drinking boiled coffee (5). The lower serum values of γ-glutamyltransferase (γ-GT) among habitual consumers of boiled coffee (5–7) are also due to consumption of cafestol and kahweol (5).

Cafestol and kahweol are largely retained by a paper filter (8, 9). Scandinavian-type boiled coffee and other types of turbid coffee brews, such as Turkish coffee (brewed by boiling powdery coffee grounds with water), French press (also known as plunger or cafetiere coffee) coffee, and espresso coffee contain diterpenes in both oil droplets and in floating coffee bean particles (8, 10, 11). Diterpenes in coffee oil affect serum lipids and liver enzymes (5). Floating coffee fines may add considerably to the intake of cafestol and kahweol from such brews. However, it is unknown to what degree cafestol and kahweol are absorbed from grounds.

We therefore studied particle contents of turbid coffee brews and changes in serum lipid concentrations, creatinine, and ALT and γ-GT activities in volunteers after consumption of spent coffee grounds.

Subjects and methods

Subjects

Approval for the studies was obtained from the Human Ethics Committee of the department. Subjects were recruited by personal approach. The study protocol was explained to them before they gave their written informed consent. None suffered from glucosuria or proteinuria, and none reported a history of gastrointestinal, liver, or kidney diseases. One woman withdrew from study 2 in the first week because of gastrointestinal discomfort. All other participants completed study 1 (n = 14) or study 2 (n = 15) successfully. They were mostly young, lean, and nonsmoking (**Table 1**). They consumed no or only moderate amounts of alcohol and coffee and did not take medications affecting serum lipids or liver enzymes, the concentrations of which were all within normal limits.

Particle content of coffee brews

Brews were prepared with coarse (Roodmerk; Douwe Egberts, Utrecht, Netherlands), fine (Café Honesta; Marvelo BV, Zaandam, Netherlands), very fine (Espresso Piazza; Douwe Egberts, Utrecht, Netherlands), or powdery grounds (Misr Cafe; Misr CAFECO, Ramadan City, Egypt) (12).

Scandinavian boiled coffee was brewed with 10 min boiling and 5 min settling time. French press coffee was prepared by pouring boiling water onto grounds in a 1-L glass jug (Bodum AG, Triengen, Switzerland), and pushing down the metal

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TABLE 1
Baseline characteristics of the participants

	Study 1 (n = 6 M, 8 F)	Study 2 (n = 9 M, 6 F)
Age (y)	24 ± 3[1]	26 ± 5
Body mass index[2]	21.8 ± 2.1	22.0 ± 2.3
Number of oral contraceptive users	2	2
Number of smokers	2	3
Daily coffee consumption		
(L)	≈0.75 ± 0.5	≈0.75 ± 0.5
(cups)	3 ± 2	3 ± 2
Weekly alcohol consumption (glasses)	7 ± 7	4 ± 3

[1] $\bar{x}$ ± SD.
[2] In kg/m^2. Measured in subjects without shoes or heavy clothing.

screen strainer (plunger) after 5 min incubation. Percolated coffee was prepared by recirculating boiling water for 20 min with a household percolator. Espresso coffee was prepared with a household espresso machine (Espresso Duo; Philips, Eindhoven, Netherlands), and mocha coffee with an aluminum mocha maker (Marimba; ABC, Crusinallo, Italy). Two different Middle Eastern brews were prepared. For Israeli mud coffee (13), grounds were mixed with boiling water in a cup and allowed to settle for 5 min. For Turkish/Greek coffee (12), grounds were added to boiling water in a traditional Turkish brewing device (ibrik, 100 mL). When a foam had formed, the heat was turned off and the brew decanted. Drip-filtered brews were prepared in an electric coffee maker (Philips, Eindhoven, Netherlands) with a paper (Melitta, Gorinchem, Netherlands), cotton, nylon, or gold filter (Swissgold, Elfo Ag Sachseln, Sachseln, Switzerland). All brews were prepared in fourfold.

Ten milliliters of each unfiltered brew, espresso, mocha, or French press brew was centrifuged at 1580 × g at room temperature for 5 min (Centaur 2, MSE, Crawley, UK) and the lipid-containing upper layer was washed away with 1 mL hexane. The tube was then dried at 105 °C and weighed. For drip-filtered and percolated brews, 200-mL samples were centrifuged in 250-mL containers at 19 200 × g (Highspeed 18; MSE). The lipid-containing upper layer was washed away with 25 mL hexane. The particle precipitate was then collected by filtration over paper (Schleicher & Schuell, Dassel, Germany), dried at 105 °C, and weighed.

Preparation of the grounds

Medium-roasted Mexican Arabica beans of one batch (Simon Levelt, Amsterdam) were used for both studies. They were ground in a beaker with a rotation blade (KM75; Krups, Solingen, Germany). For study 1, fine grounds were obtained by grinding beans to pass a 0.5-mm metal sieve (Retsch, Haan, Germany). For study 2, coarsely ground coffee was obtained by collecting grounds passing a 1.4- but not a 1.0-mm sieve, whereas grounds passing the 1.0-mm sieve were ground to pass a 0.5-mm metal sieve to obtain fine grounds.

Spent coffee grounds were prepared twice a week by mixing ground coffee beans with boiling water (70 g/L), boiling them for 5 min, and allowing them to settle for 5 min. The spent grounds were collected on a 75-μm metal sieve (Retsch, Haan, Germany) and allowed to drain for 30 min before being divided into daily portions.

We analyzed cafestol and kahweol contents in coffee grounds of every brewing session for study 1, and in one portion of study 2. Lipids were saponified with 5 mol ethanolic KOH/L, and 5,α-cholestane was added as an internal standard. The free diterpene alcohols were extracted with diisopropylether, and analyzed as trimethylsilylethers on a Hewlett-Packard 5890 series II gas chromatograph (Avondale, PA). Authenticity and purity of the peaks were verified on a Hewlett-Packard G1019A mass spectrometer.

Hypotheses and designs

We examined the effect of fine grounds in a randomized controlled parallel study (study 1), and studied the influence of particle size by giving either fine or coarse grounds in a crossover study (study 2).

Study 1. The hypothesis to be tested was that daily ingestion of 8 g coffee grounds elevates serum cholesterol and ALT activity. Calculations showed that we would need six subjects per group to detect a difference in serum cholesterol of 0.50 mmol/L with a power of 90%, and seven to detect a difference in ALT of 20 U/L (α = 0.05).

During the run-in period of 14 d, subjects consumed 125 mL hopjes-caramelvla, a commercially available, sweet-flavored dairy dessert providing 4.8 g fat (2.5 g saturated and 1.3 g monounsaturated fatty acids) and 12.5 mg cholesterol/d. Venous blood samples were taken from each subject on days −3 and 0.

During the test period of 21 d, both groups consumed 125 mL hopjes-caramelvla/d. The experimental group mixed fine spent coffee grounds just before consumption with the hopjes-caramelvla, which masked the bitter taste of the grounds fairly well. Subjects were provided with grounds twice a week and were requested to store them at 4 °C. They were free to choose the time of consumption, but they had to consume them every day at the same time. Consumption of the grounds was not allowed within 0.5 h after subjects had taken any food or drink except water.

Compliance was monitored by asking the subjects to report the time of consumption of the grounds daily in a special diary. They were instructed to maintain their usual dietary and living habits and to report deviations from it. Subjects were asked to restrict coffee use during the experiment to paper-filtered coffee with a daily maximum of 1.5 L (6 cups). Tea was allowed freely. They also kept daily records of coffee and alcohol consumption and medication use. Venous blood samples were taken on days 18 and 21, and 59 d after the experiment (day 94).

Study 2. We hypothesized that serum cholesterol and serum ALT would be higher on fine grounds than on coarse grounds. The study was designed to detect a difference between the two treatments in serum cholesterol of 0.20 mmol/L and in serum ALT of 10 U/L with a power of 90% (α = 0.05).

Baseline blood samples were drawn on days −4 and 0. Subjects were randomly assigned into two groups and consumed either fine or coarse grounds for 11 d. On day 11 blood samples were taken. No grounds were consumed on days 12, 13, or 14. Then treatments were switched. Final blood samples were drawn on days 25 and 28. Serum levels were checked in 14 subjects 99 d after the experiment (day 127). Other requirements and restrictions were similar to those for study 1.

Blood assays

Blood samples were taken after an overnight fast. Sera were obtained by centrifugation, stored at −80 °C, and analyzed within one run. Sera obtained postexperimentally were analyzed separately. Sera were analyzed enzymatically for total cholesterol (14) and triglycerides (15). Mean bias for control sera provided by the Centers for Disease Control (Atlanta) was −2% for total cholesterol and 4% for triglycerides. The CV within runs ranged from 0.7% to 2.1%. Creatinine was measured with a modified Jaffé method by using a Spectrum kit (Abbott Laboratories, North Chicago, IL) (16). Serum activities of ALT, aspartate aminotransferase (AST) (17), γ-GT (18), and alkaline phosphatase (19) were measured at 37 °C by using Abbott Spectrum reagents. The mean bias for Monitrol control sera (Baxter Dade AG, Düdingen, Switzerland) ranged from −0.3% to 1%. The CV within runs ranged from 0.8% to 10%. Upper limits of normal were 53.5 U/L for ALT activity, 39.7 U/L for AST activity, 92 U/L for alkaline phosphatase activity, and 63 (men) and 35 (women) U/L for γ-GT activity.

Statistics

Study 1. Effects of grounds consumption were investigated by subtracting the mean of the two baseline values (days −3 and 0) from the mean of the values on grounds at the end of the trial (days 18 and 21). Differences in group means of changes were tested against 0 by using an unpaired, one-tailed *t* test.

Study 2. Effects of particle size were analyzed by comparing values obtained on days 11 and 25, ie, after 10 d consumption of either type of grounds; *t* tests showed period effects for all serum variables ($P < 0.05$) except triglycerides (20). However, *t* tests for equality of carryover effects from the first into the second period (20) showed similar carryover between the two groups for all variables ($P = 0.10$). Therefore, values after consumption of coarse grounds were subtracted from values after fine grounds, irrespective of sequence, and differences were tested against 0 with a one-tailed *t* test.

Results

Particle content of coffee brews

Unfiltered coffee types contained the highest amount of floating fines, ranging from 2 g dry wt/L Scandinavian coffee to ≈5 g/L for Middle Eastern coffee brews (**Table 2**). French press coffee contained 1.5 g/L and other unfiltered coffee types contained less. Use of a paper filter led to negligible amounts of coffee fines.

TABLE 2
Particle content of various coffee brews as consumed

Coffee type	Brewing strength *g/L water*	Grind	Particle content[1] *g dry wt/L*
Unfiltered			
Scandinavian boiled	80	Coarse	2.1 ± 0.5
Israeli mud (13)	80	Powdery	5.0 ± 1.2
Turkish/Greek	80	Powdery	5.3 ± 1.8
Other			
French press	50	Coarse	1.5 ± 0.2
Espresso	150	Very fine	0.8 ± 0.1
Mocha	100	Very fine	1.2 ± 0.4
Percolated	50	Coarse	0.4 ± 0.0
Drip filtered			
Paper filter	50	Fine	0.1 ± 0.0
Nylon filter	50	Fine	0.4 ± 0.0
Gold filter	50	Fine	0.6 ± 0.1
Cotton filter	50	Fine	0.3 ± 0.1

[1] $\bar{x}$ ± SD.

Effect of coffee grounds on serum lipids and liver enzymes

The 29 of 30 subjects who completed either of the studies successfully reported only minor complaints. Two subjects reported flatulence during the first week of treatment. Hyperactivity was reported by two subjects during the first days, even though caffeine was probably largely absent from the grounds (11).

Study 1. Diaries kept by the subjects showed that all 147 daily portions of grounds had been consumed. The dry weight of the grounds portions was 7.8 ± 0.2 g/d (x ± SD). The grounds provided 39 ± 4 mg cafestol and 49 ± 5 mg kahweol/d ($n = 5$). After 21 d of grounds consumption, participants showed a mean rise in serum cholesterol of 0.65 mmol/L (95% CI 0.41, 0.89 mmol/L) relative to the control group (**Table 3, Fig 1**). An increase in serum cholesterol was seen in each of the subjects consuming coffee grounds, ranging from 0.29 to 1.09 mmol/L. The mean rise in ALT activity relative to control subjects was 18 U/L (95% CI

TABLE 3
Study 1: changes in values of serum lipids, liver enzymes, and creatinine in healthy volunteers consuming hopjes-caramelvla (control group) or hopjes-caramelvla with 7.8 g dry wt of fine spent coffee grounds (treatment group)/d for 21 d, and differences between the changes

Index[1]	Control group ($n = 7$)	Treatment group ($n = 7$)	Difference between groups (95% CI)	*P*
Cholesterol (mmol/L)[2]	0.01 ± 0.23[3]	0.66 ± 0.24	0.65 (0.41 to 0.89)	<0.001
Triglycerides (mmol/L)[4]	0.06 ± 0.27	0.36 ± 0.34	0.30 (−0.02 to 0.62)	0.05
ALT (U/L)	3 ± 4	21 ± 19	18 (4 to 32)	0.02
γ-GT (U/L)	0 ± 1	−2 ± 2	−3 (−4 to −1)	0.01
AST (U/L)	2 ± 3	9 ± 9	7 (−0 to 14)	0.05
Alkaline phosphatase (U/L)	0 ± 3	−6 ± 7	−6 (−12 to −1)	0.03
Creatinine (μmol/L)	1 ± 7	−5 ± 6	−6 (−13 to 1)	0.06

[1] ALT, alanine aminotransferase; γ-GT, γ-glutamyltransferase; AST, aspartate aminotransferase.
[2] To convert to mg/dL, multiply by 38.67.
[3] $\bar{x}$ ± SD.
[4] To convert to mg/dL, multiply by 88.54.

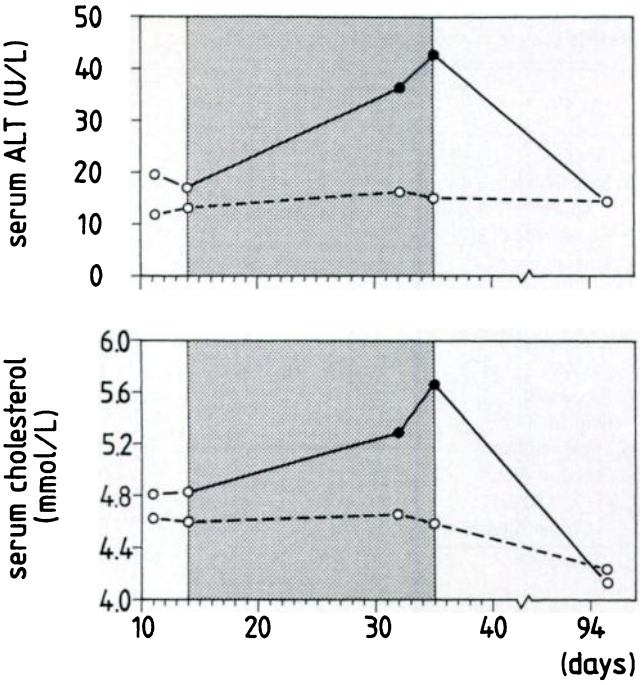

FIG 1. Study 1: serum cholesterol and alanine aminotransferase concentrations in two groups of seven healthy volunteers at baseline (○), after daily consumption of 125 mL of hopjes-caramelvla with (●) or without (○) 7.8 g of fine spent coffee grounds, and 59 days after the experiment (○). The horizontal black bar indicates the period during which grounds were administered.

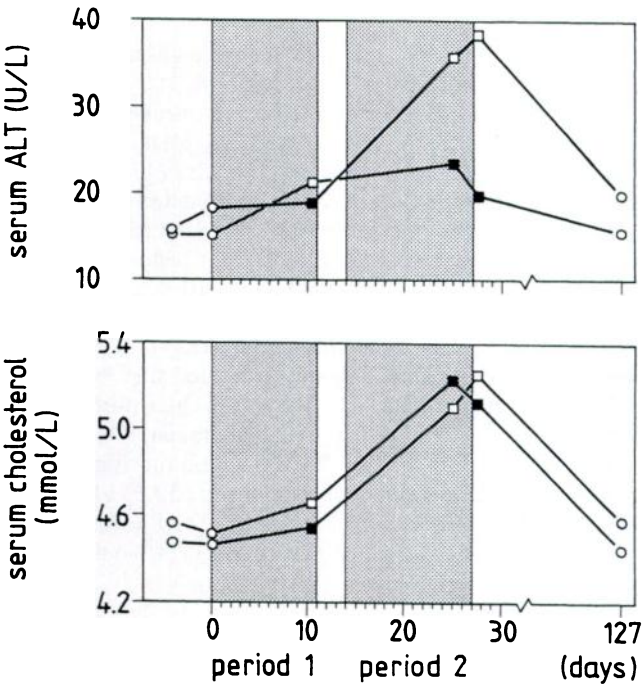

FIG 2. Study 2: serum cholesterol and alanine aminotransferase concentrations in 15 healthy volunteers at baseline (○), after daily consumption of 6.6 g fine (□) or 7.1 g coarse (■) grounds, and 99 d after the experiment (○) (n = 7 or 8 per group). The horizontal black bars indicate the periods during which grounds were administered.

4–32 U/L) or 0.3 times our upper limit of normal. The treatment group also showed higher values of serum triglycerides and serum AST, and lower values of γ-GT, alkaline phosphatase, and creatinine ($P < 0.10$). Fourteen weeks after the trial serum values had returned to baseline level.

Study 2. Diaries showed that only one of the 360 daily portions of grounds had not been consumed. Dry weights of the grounds portions of the seven brewing sessions were 7.1 ± 0.3 g/d for coarse grounds and 6.6 ± 0.5 g/d for fine grounds. The coarse coffee grounds provided 37 mg cafestol and 54 mg kahweol and the fine grounds 48 mg cafestol and 56 mg kahweol/d (n = 1). Serum cholesterol concentrations showed a similar response for both treatment sequences (**Fig 2**). Mean concentrations were 4.9 mmol/L after consumption of both fine and coarse grounds (**Table 4**). Mean ALT activity in serum was 29 U/L after consumption of fine vs 21 U/L after coarse grounds (p for difference 0.02). Serum values of triglycerides and AST were higher, and those of γ-GT lower after consumption of fine vs coarse grounds ($P < 0.05$).

Discussion

Particle content of coffee brews

Scandinavian boiled coffee, the coffee type most commonly associated with raised cholesterol concentrations (1–4), contained 2.1 g coffee fines/L. If we assume that the diterpene content of these coffee fines is similar to that of the fine grounds used in our trial (39 mg cafestol and 49 mg kahweol in 7.8 g fines), then the grounds in boiled coffee would

TABLE 4
Study 2: serum lipids, liver enzymes, and creatinine of 15 healthy volunteers after daily consumption of 6.6 g fine and 7.1 g coarse grounds for 10 d each in a crossover design[1]

Index[1]	Fine grounds	Coarse grounds	Difference (fine − coarse)	P
Cholesterol (mmol/L)[2]	4.89 ± 0.73[3]	4.86 ± 0.65	0.03 ± 0.71	0.43
Triglycerides (mmol/L)[4]	1.31 ± 0.54	1.01 ± 0.45	0.31 ± 0.51	0.02
ALT (U/L)	29 ± 18	21 ± 9	8 ± 14	0.02
γ-GT (U/L)	13 ± 4	15 ± 4	−2 ± 1	<0.001
AST (U/L)	26 ± 7	22 ± 5	5 ± 4	<0.001
Alkaline phosphatase (U/L)	63 ± 18	63 ± 15	0 ± 6	0.41
Creatinine (μmol/L)	86 ± 13	85 ± 13	0 ± 8	0.42

[1] ALT, alanine aminotransferase; γ-GT, γ-glutamyl transferase; AST, aspartate aminotransferase.
[2] To convert to mg/dL, multiply by 38.67.
[3] $\bar{x}$ ± SD.
[4] To convert to mg/dL, multiply by 88.54.

provide 11 mg cafestol and 13 mg kahweol/L brew. This is 22% of the total amount of 108 mg diterpenes/L (8). Values for Middle Eastern brews, known variously as Turkish, Greek, Arab, or Israeli mud, were even higher. Middle Eastern boiled coffee contained 5.3 g fines/L, which would provide 27 mg cafestol and 33 mg kahweol, accounting for about half the total amount of diterpenes present in Middle Eastern brew (8). Obviously, diterpene contents in such turbid brews will be affected strongly by the amount of particles decanted with the brew; therefore, an analytical method that includes the contribution of ingested coffee fines to the total amount of diterpenes in the brew is essential.

Effect of coffee grounds on serum lipids and enzymes

Study 1. In this parallel study, daily ingestion of 8 g coffee grounds significantly raised serum cholesterol and ALT concentrations compared with those of control subjects. These elevations were similar to those caused by boiled coffee, coffee oil, or similar amounts of cafestol and kahweol as pure compounds dissolved in oil (5). In the present study a mean daily intake of 39 mg cafestol and 49 mg kahweol with coffee grounds resulted in a rise in serum total cholesterol of 0.65 mmol/L (26 mg/dL). We estimated earlier that each extra 2 mg cafestol ingested in oily solutions increases serum total cholesterol by 0.03 mmol/L (1 mg/dL) (5). If cafestol absorption from coffee oil and boiled grounds is equal, the predicted rise for the present study would be 0.52 mmol/L (20 mg/dL). This is only slightly lower than the observed rise. In our previous studies serum ALT concentrations rose on average by 1 IU/L for every 2 mg cafestol ingested (5). If cafestol in bean particles were as available as cafestol in oil, then the boiled grounds should have raised serum ALT by 20 U/L. The observed rise was 18 U/L. These results suggest that cafestol availability from ingested grounds is comparable with that from coffee oil. Furthermore, the changes in the other liver enzymes—γ-GT, alkaline phosphatase, and AST—and the changes in serum triglycerides and creatinine were all in the same directions as in previous studies (5).

The duration of grounds consumption was 3 wk. In previous studies (5, 9) the effects of cafestol and kahweol on serum cholesterol and ALT were maximal after ≈4 wk and were stable thereafter. Thus, any underestimation due to treatment duration may be considered small.

Study 2. The effect of particle size on the availability of cafestol and kahweol was examined in a crossover study. Comparison of the two particle sizes, however, was complicated by the unexpectedly different cafestol content of the samples: 48 mg cafestol in fine vs 37 mg in coarse grounds. Later experiments (unpublished data) confirmed that grinding and sieving of beans led to fractionation of lipid material, with fine grounds having a higher cafestol content. The larger effect of fine grounds on ALT and γ-GT activities and triglycerides could therefore be due to their higher cafestol content rather than to particle size. However, despite the difference in cafestol, response of serum cholesterol was highly similar for both treatments (Fig 2). Possibly, serum cholesterol is more sensitive to the effects of cafestol than is serum ALT. Furthermore, kahweol could have lessened the difference between the two groups in this study, because the grounds were comparable in kahweol content (56 mg kahweol in fine grounds and 54 mg in coarse grounds). The separate effects of cafestol and kahweol on blood lipids and indexes of liver and kidney function still have to be established. However, it would appear that an appreciable amount of cafestol and kahweol is already absorbed from the coarse grounds.

Conclusion

Cafestol and kahweol from coffee grounds raise serum cholesterol and ALT activity similar to cafestol and kahweol from boiled coffee or coffee oil. Because appreciable amounts of diterpenes are carried by floating coffee bean particles in turbid brews, especially of the Middle Eastern types, analyses of these brews should include the contribution of fines. Finally, frequent ingestion of coffee bean particles or of grounds with turbid coffee brews should be avoided.

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