Levels of the Cholesterol-Elevating Diterpenes Cafestol and Kahweol in Various Coffee Brews

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The coffee diterpenes cafestol and kahweol raise serum cholesterol in humans. Each 10 mg of cafestol consumed per day elevates cholesterol by 5 mg/dL (0.13 mmol/L). Diterpene levels in various coffee brews were examined. Scandinavian boiled coffee contained (mean \pm SD) 3.0 \pm 2.8 mg, French press coffee 3.5 \pm 1.2 mg, and Turkish/Greek coffee 3.9 \pm 3.2 mg of cafestol per cup. Consumption of five cups per day of any of these coffee types could thus elevate serum cholesterol by 8–10 mg/ dL. Italian espresso coffee contained 1.5 \pm 1.0 mg of cafestol per cup, five cups theoretically raising cholesterol by 4 mg/dL. Brewing time had little effect of diterpenes. Brewing strength increased diterpenes in boiled, French press, and espresso coffee but not in Turkish/Greek coffee. Diterpenes in instant, drip filtered, and percolated brews were negligible. Regular and decaffeinated coffees had similar diterpene contents. High chronic intake of French press coffee or Turkish/Greek coffee could increase serum cholesterol and thus coronary risk similar to that reported previously for Scandinavian boiled coffee.

Keywords: Coffee; cafestol; kahweol; brewing method; serum cholesterol; coronary disease

INTRODUCTION

Scandinavian-type boiled coffee raises serum cholesterol in man (Aro, 1993; Thelle et al., 1987). The diterpenes cafestol and, possibly, kahweol are responsible for this effect (Heckers et al., 1994; Weusten-van der Wouw et al., 1994). Additionally, cafestol and kahweol affect liver function; they increased the activity of alanine aminotransferase and depressed that of γ -glutamyltransferase in serum. In controlled trials, serum cholesterol rose by about 1 mg/dL (0.03 mmol/L) and alanine aminotransferase by about 1 unit/L for each extra 2 mg of cafestol ingested (Weusten-van der Wouw et al., 1994). Levels of cafestol and kahweol in various coffee brews could be used to predict their capacity to affect lipoprotein metabolism and liver function and are thus an important health issue.

Cafestol and kahweol represent the major part of the unsaponifiable lipid fraction in coffee beans. They are mainly present as fatty acid esters, but small amounts of free alcohols also occur. The total diterpene content is 1.3% w/w in green beans of *Coffea arabica* (commonly called Arabica beans) and 0.2% in beans of *Coffea canephora* (commonly called Robusta beans) (IARC Working Group, 1991). Robusta beans are almost devoid of kahweol (Viani, 1988) but contain a third diterpene—16-O-methylcafestol—which is absent in Arabica beans (Speer and Mischnick, 1989). Other diterpenes, such as decomposition products of cafestol and kahweol, are present in very low quantities and are therefore unlikely to affect serum lipids and liver enzymes substantially.

Brewing releases oil droplets containing diterpenes from ground coffee beans. They are retained by a paper filter (Ratnayake et al., 1993), which explains why paper-filtered coffee shows no (Ahola et al., 1991; van



Figure 1. Brewing principles for percolated, French press (cafetiere or plunger), and mocha coffees.

Dusseldorp et al., 1991) or only little (Fried et al., 1992) effect on serum cholesterol. With espresso and mocha coffee, and with French press coffee, also known as plunger or cafetiere coffee, lipids readily pass the metal filter (Ratnayake et al., 1993) and the hypercholesterolemic diterpenes may thus be removed less efficiently from the brew. Other brews, such as Scandinavian boiled coffee and Middle Eastern coffee types, are decanted directly from the boiling state into the cup without applying a filter at all (IARC Working Group, 1991).

We report diterpene contents of various coffee brews and their potential impact on serum cholesterol levels.

EXPERIMENTAL METHODS

Collection of Field Samples. Boiled coffee was collected from regular consumers in Norway and Finland (n = 14); Turkish/Greek coffee was collected from Turkish and Greek restaurants operated by immigrants in the Netherlands (n =7) and from retail outlets in Greece and Egypt (n = 4). Espresso was from bars and restaurants in Italy (n = 10), Spain (n = 2), Switzerland (n = 4), and the Netherlands (n =15). French press coffee (Figure 1) was obtained from consumers in the Netherlands (n = 5). Brews were poured into plastic containers so as to mimic the amount of the brew that is consumed from the cup and frozen at -20 °C. Cup size was defined as the amount poured into the container.

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Thirteen regular and 6 decaffeinated instant (soluble) coffees were analyzed. Instant brands included Folgers red and green, Tasters Choice red and green, Sanka To Go decaffeinated (decaf), and Maxwell House (United States), Nescafé Espresso, Nescafé Cappuccino, Nescafé Classico roodmerk and decaf, Nescafé Cap Colombie, Douwe Egberts Moccona roodmerk and decaf, Douwe Egberts Moccona Espresso, and Koffie Hag (The Netherlands), MISR Cafe and Mister Cafe (Egypt), Nescafé Classic (South Africa), and Africafe (Tanzania).

Twenty regular and 5 decaffeinated brands of roast and ground coffee beans were analyzed. Brands included Hills Brothers red and green, Folgers Mountain Grown and decaf, Maxwell House Master Blend, and Maxwell House decaf (United States), Albert Heijn Perla, Van Nelle Supra, Cafe Idee, Douwe Egberts Kleintje Koffie and decaf, Douwe Egberts Piazza Espresso, and douwe Egberts roodmerk (The Netherlands), Jacobs Espresso Mastro Lorenzo and decaf and Jacobs Espresso Medaille d'Or (Germany), Rombouts Espresso (Belgium), Evergood Kaffe, and Friele Frokost Kaffe (Norway), Paulig Juhla Mokka (Finland), Elite ALAD IN (Israel), MISR Cafe (Egypt), Kopi Tora Bika (Indonesia), and Yambone coffee and Banja coffee (Malawi).

To examine the influence of roasting, we roasted samples of 250 g of Mexican Arabica beans (Cafe Organico, Simon Levelt, Amsterdam, The Netherlands) with a Type B3 sample roaster (Probat, Germany) for 4.5 (light roast), 5.0 (medium), and 5.8 min (dark).

Preparation of Brews in the Laboratory. We studied the effect of brewing method and duration and of the ratio of coffee grounds to water (brewing strength) under laboratory conditions. We used one large batch of medium-roasted Mexican Arabica beans (Simon Levelt, Amsterdam) for all brews. Beans were ground with a commercial coffee bean grinder (La Pavoni, Italy) to coarse or fine grind. Particle size distribution (w/w) as determined with metal sieves (Haan, Retsch, Germany) was, for coarse grounds, 25% larger than 1.0 mm, 50% between 0.6 and 1.0 mm, and 25% between 0.42and 0.6 mm; and, for fine grounds, 17% larger than 0.6 mm, 26% between 0.42 and 0.6 mm, 48% between 0.2 and 0.42 mm, and 9% between 0.075 and 0.2 mm. Powdery grounds were obtained by pulverizing fine grounds in a beaker with rotation blade (Krups KM75, Solingen, Germany). Ten percent of the powdery ground material was larger than 0.42 mm, 38% between 0.2 mm and 0.42 mm, 34% between 0.075 mm and 0.2 mm, and 18% smaller than 0.075 mm.

Brews were prepared in triplicate and stored at -20 °C for a maximum of 3 months.

Boiled Coffee Types. "Scandinavian" boiled coffee was brewed in the laboratory by boiling coarsely ground beans with water, followed by 5 min of settling time. The liquid was decanted until particles of the sediment started to come with the fluid.

We examined two types of Middle Eastern coffee. "Turkish/ Greek" coffee was prepared by bringing powdery grounds to a foamy boil with water in a domestic Turkish brewing pot. Then the brew was decanted into a cup. "Israeli mud" coffee (Kark et al., 1985) was prepared by pouring boiling water onto powdery grounds in a cup. For both coffee types, the contents of each cup were decanted again after having settled for 5 min so as to mimic usual consumption.

Other Coffee Types. French press coffee (Figure 1) was brewed by pouring boiling water onto coarsely ground beans in a glass plunger pot of 1 L (Bodum AG, Triengen, Switzerland) and pushing down the metal screen strainer (plunger) after 5 min.

Espresso coffee was brewed with fine grind. We compared espresso brewed with two types of espresso machines for household use from Philips (Eindhoven, The Netherlands) and one from Krups (Solingen, Germany), using three extraction volumes (50, 100, and 200 mL) and three roasting grades (light, medium, and dark).

Mocha coffee was brewed with fine grind in an aluminum mocha-maker (Marimba, ABC, Crusinallo, Italy) (Figure 1).

Percolated coffee was prepared by recirculating boiling water for 20 min through coarse grounds using a household percolator of 1 L (Figure 1). Drip-filtered brews were prepared with fine grind in an electric coffee maker (Ebony 053, Moulinex, France) with a paper bag filter (Melitta, Gorinchem, The Netherlands) or with a cotton (Bean Bag, United States), nylon (Prestige, France); or gold-plated (Swissgold, Elfo Ag Sachseln, Sachseln, Switzerland) "permanent" filter.

Analysis of Diterpenes. Coffee brew was heated to 60-90 °C under continuous stirring, and 4 mL was pipetted into a screw-capped tube. Two milliliters of 5α -cholestane (Pierce, Eurochemie, Oud-Beijerland, The Netherlands, no. 17060) in ethanol was added as internal standard. Its concentration in ethanol was 25, 200, or 500 mg/L if cafestol in the brew was expected to be <12.5, between 12.5 and 100, or >100 mg/L, respectively.

Lipids were extracted by adding 4 mL of diisopropyl ether (Merck, Darmstadt, Germany, no. 867), shaking for 10 min at 250 oscillations/min in a mechanical shaker (Swip SM25, Buehler, Switzerland), and centrifuging for 5 min at 3000 rpm (Centaur 2, MSE, England). The ether phase was taken off. The water phase was re-extracted once with 4 mL and once with 2 mL of diisopropyl ether. The combined extracts were evaporated at 45 °C with nitrogen and redissolved in 200 μ L of absolute ethanol (Merck, no. 983). One milliliter of 0.5 M potassium hydroxide in ethanol was added, and the solution was saponified by incubating for 15 min in a water bath at 80 °C.

Beans or commercial ground coffees were ground in a beaker with a rotation blade (Krups, KM75, Solingen, Germany) to pass a 600 μ m sieve (Retsch, Haan, Germany). Then, 100–200 mg of grounds was combined with 1 mL of 5 α -cholestane in ethanol (1 g/L) and 1 mL of 5 M potassium hydroxide in ethanol and saponified for 60 min in a shaking water bath at 80 °C.

After saponification, the procedure was identical for brews and grounds. One milliliter of demineralized water was added, and the water phase was extracted three times with 2 mL of diisopropyl ether. Three milliliters of demineralized water was added to the combined solvent fractions, and the mixture was shaken for 10 min at 250 oscillations/min and centrifuged for 5 min at 3000 rpm. The ether phase was dried at 45 °C with nitrogen, and the residue was redissolved into 1.5 mL of diisopropyl ether. The solution was transferred into a 2 mL sampler vial and dried at 45 °C with nitrogen, and the residue was dissolved in 0.5 mL of dried pyridine (Merck, no. 7463). Then 150 μ L of a 2:1 (v/v) mixture of hexamethyldisilazane (Pierce, no. 84770) and trichloromethylsilane (Pierce, no. 88530) was added. After 30 min at ambient temperature, excess pyridine was removed under a stream of nitrogen, and 1.0 mL of HPLC grade hexane (Rathburn Chemicals Ltd., Scotland) was added. The vial was shaken and centrifuged, and the supernatant was diluted with hexane 8 times if cafestol in the brew was expected to be between 12.5 and 100 mg/L and 20 times if cafestol was >100 mg/L. The sample was transferred to a clean vial, and 1.0 μ L was injected splitless into a Hewlett-Packard 5890 Series II gas chromatograph (Avondale, PA) equipped with a 25 m \times 0.22 mm fused silica CP Sil5CB column (Chrompack, Middelburg, The Netherlands). The initial oven temperature was 70 °C for 2.5 min followed by a rise to 200 °C at a rate of 40 °C/min. After 10 min, the temperature was raised to 235 °C at a rate of 6 °C/ min and then to 285 °C at a rate of 30 °C/min, at which it was held for 6.75 min. Other conditions were as follows: carrier gas, hydrogen; pressure, 100 kPa; makeup gas, nitrogen; splitless injection after 2.5 min with a injector purge flow of 100 mL/min at 300 °C; flame ionization detector temperature. 305 °C. A typical gas chromatogram for a Turkish/Greek coffee sample is shown in Figure 2. The system was calibrated with a mixture of authentic cafestol, kahweol, and 16-O-methylcafestol provided by Nestec Ltd., Switzerland. Authenticity and purity of the peaks were verified on a Hewlett-Packard G1019A GC mass spectrometer. We also detected small amounts of decomposition products of cafestol and kahweol (Figure 2), formed by loss of the 16-OH function by dehydration during processing.

The coefficients of variation for a control pool of boiled coffee were 3.0% within and 6.3% between runs over a 6-month

Table 1. Mean Levels \pm SD (Ranges) of Cafestol, Kahweol, and 16-O-Methylcafestol in Coffees Collected from Bars andRestaurants and from Regular Consumers in a Range of Countries (cf. Experimental Methods)

type of coffee	$cafestol^a$	ka hweol ^a	16-O-methylcafestol
Scandinavian boiled $(n = 14)$	$3.0 \pm 2.8 \ (0.8 - 12.1)$	$3.9 \pm 3.4 (1.1 - 14.6)$	$0.0 \pm 0.0 (0.0 - 0.1)$
Turkish/Greek $(n = 11)$	$3.9 \pm 3.2 (0.5 - 10.0)$	$3.9 \pm 3.9 (0.1 - 10.7)$	$0.5 \pm 0.6 (0.0 - 1.4)$
French press $(n = 5)$	$3.5 \pm 1.2 (2.3 - 5.5)$	$4.4 \pm 2.1 (2.6 - 8.0)$	$0.1 \pm 0.1 (0 - 0.2)$
espresso			
Italy $(n = 10)$	$1.5 \pm 1.0 \ (0.2 - 2.9)$	$1.8 \pm 1.3 (0.2 - 3.9)$	$0.1 \pm 0.1 (0.0 - 0.3)$
other countries $(n = 21)$	$1.2 \pm 0.9 \ (0.0 - 3.1)$	$1.4 \pm 1.1 (0.0 - 3.9)$	$0.1 \pm 0.1 (0.0 - 0.3)$

 a Values are given in terms of mg of free diterpene alcohols per cup. For brews collected form bars and restaurants, cup size was defined as the amount of brew served per cup. For brews of Scandinavian and French press coffee made at home by regular consumers, cup size was 150 mL.

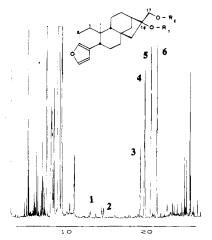


Figure 2. Gas chromatogram of silyl derivatives of coffee diterpenes in a Turkish/Greek coffee sample. Peaks: decomposition products of cafestol and kahweol (1, 2), 16-O-methylcafestol (3), kahweol (4), cafestol (5), and 5α -cholestane (6). Other peaks represent mostly free fatty acids and phytosterols. For further details, see Experimental Methods. The insert gives the chemical structure of the coffee diterpenes: cafestol (R₁ = H, R₂ = H), kahweol (R₁ = H, R₂ = H). and 16-O-methylcafestol (R₁ = CH₃, R₂ = H).

period for cafestol and 2.9% and 5.2%, respectively, for kahweol. Recoveries of cafestol and kahweol (mean \pm SD) were 102.2 \pm 2.3% and 100.3 \pm 2.5%, respectively, if added in the form of coffee oil to paper-filtered coffee (n = 6), and 100.1 \pm 3.4% and 99.3 \pm 3.2%, respectively, if added as diterpene-containing coffee grounds (n = 6). Measurements were linear over the range of 0.01-40 mg/100 mL of brew.

RESULTS

Field Samples. Scandinavian boiled and Turkish/ Greek coffees showed high variability in diterpene levels. They ranged from as low as 1 mg to more than 10 mg of cafestol per cup. On average, they contained 3-4 mg of cafestol per cup. French press coffees contained 3.5 mg of cafestol per cup of 150 mL (range 2.3-5.5 mg). Espresso coffees ranged from 0 to 3.1 mg per cup (Table 1).

Instant coffees on average contained 0.2 mg (range 0-0.6 mg) of cafestol per cup prepared with 2 g of soluble granules for both regular and decaffeinated coffees (Figure 3). Mean levels of cafestol, kahweol, and 16-O-methylcafestol were 486, 469, and 34 mg/100 g of regular coffee grounds (n = 20) and 485, 411, and 44 mg/100 g of decaffeinated coffee grounds (n = 5), respectively (Figure 3). Roasting did not reduce diterpenes in Arabica beans (Figure 4).

Brews Prepared in the Laboratory. The Arabica beans we used contained 573 mg of cafestol and 736 mg of kahweol per 100 g. The ratio of cafestol to kahweol was the same in beans and brews, indicating that they were extracted to the same extent.

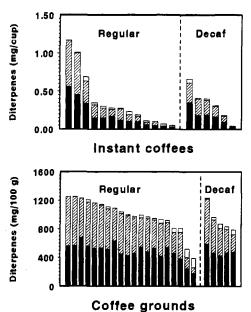


Figure 3. Levels of cafestol (solid bar), kahweol (slashed bar), and 16-O-methylcafestol (open bar) in instant (soluble) coffee granules and in commercial roast and ground coffees. For instant coffees, values are given as milligrams of free alcohols per 2 g of soluble granules, which is the amount that goes into one cup (IARC Working Group, 1991). For coffee grounds, values are milligrams per 100 g. "Regular" refers to caffeinecontaining products; "Decaf" refers to decaffeinated products.



Figure 4. Effect of roasting on levels of cafestol (solid bar) and kahweol (slashed bar) in Mexican Arabica beans, expressed as milligrams per 100 g of roasted product. Corresponding weight losses were 24.5% for light, 26.0% for medium, and 26.5% for dark roasted beans.

Boiled Coffee Types. Scandinavian-type boiled coffee of regular strength provided 4-5 mg of cafestol per cup of 150 mL (Figure 5), slightly higher than levels in field samples; however, variability in the laboratory-prepared brews was much lower. Brewing strength highly determined diterpene content; each extra 10 g of coffee grounds (equivalent to 1-2 household scoops) used per liter of water for brewing increased cafestol by 0.7 mg per cup. Duration of brewing was less important; increasing boiling time from 5 to 10 min increased cafestol and kahweol by 9% and from 5 to 30 min by 33%.

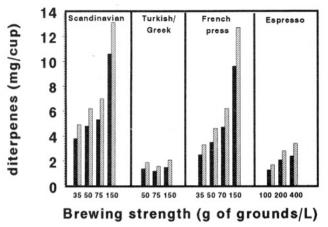


Figure 5. Influence of brewing strength (concentration of coffee grounds per liter of fresh water used for brewing) on levels of cafestol (solid bar) and kahweol (slashed bar) in Scandinavian boiled, Turkish/Greek, French press, and espresso coffee prepared in the laboratory under standard conditions. Brews were prepared in triplicate. Values are given as milligrams of free alcohols per cup of 150 mL.

Cafestol levels in Turkish/Greek coffee made in the laboratory were 1–2 mg per cup of 60 mL and were unaffected by brewing strength (Figure 5). Israeli type "mud" coffee made in the laboratory contained 0.8 ± 0.0 mg of cafestol and 1.1 ± 0.1 mg of kahweol per cup of 60 mL.

Other Coffee Types. French press coffee of regular strength provided 3-4 mg of cafestol per cup of 150 mL (Figure 5). Increasing brewing strength by 10 g/L increased cafestol by 0.6 mg per cup. Applying an incubation time of 1 instead of 5 min only decreased diterpenes by 6%.

Espresso coffee made in the laboratory contained 1–2 mg of cafestol per cup of 25 mL (Figure 5). All of the extractable diterpenes were already extracted with the first 100 mL of water that had been forced through the coffee grounds. Mocha coffee (100 g/L) contained (mean \pm SD) 1.1 \pm 0.1 mg of cafestol and 1.4 \pm 0.2 mg of kahweol per cup of 60 mL.

Percolated coffee and coffee prepared with a paper or permanent filter in an automatic drip filter machine maximally provided 0.5 mg of cafestol per cup of 150 mL. Pouring boiling water by hand on coffee grounds resulted in 2.5 ± 1.2 mg of cafestol per cup for goldplated (n = 21), 0.8 ± 0.1 mg for nylon (n = 3), and 0.1 ± 0.0 mg for paper filters (n = 3).

DISCUSSION

The major finding from this study is that Turkish/ Greek and French press, also known as plunger or cafetiere coffee, may have cafestol and kahweol levels similar to those of Scandinavian boiled coffee, the coffee type most frequently associated with elevated serum cholesterol levels.

Boiled Coffee Types. Field samples of Scandinavian and Turkish/Greek coffee varied strongly in diterpene content, probably attributable to differences in brewing strength and amount of coffee bean particles decanted with the brew. They both ranged from 1 to more than 10 mg of cafestol per cup of 150 mL, with averages of 3.0 and 3.9 mg, respectively. Five cups of either of these coffee types per day thus on average provide 15–20 mg of cafestol, which will raise serum cholesterol by about 8–10 mg/dL or 0.2–0.25 mmol/L (Weusten-van der Wouw et al., 1994) (Figure 6). Finn-



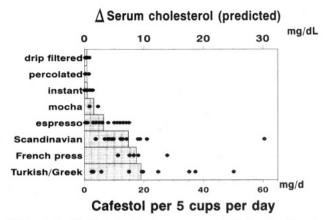


Figure 6. Predicted effect on serum cholesterol of daily consumption of five cups of various types of coffee. Black dots represent individual and bars mean values of field samples. Estimations of the rise in serum cholesterol are based on the observation of Weusten-van der Wouw et al. (1994) that every 10 mg of cafestol consumed per day raises serum cholesterol by 5 mg/dL (0.13 mmol/L).

ish men and women chronically drinking 7-9 cups of boiled coffee per day indeed had cholesterol levels 23 mg/dL higher than their peers consuming filter coffee (Pietinen et al., 1990), and Norwegians consuming 5 or more cups had levels 12 mg/dL higher (Weusten-van der Wouw et al., 1994). Thus, our figures allow a valid prediction of the effect of coffee consumption on serum cholesterol.

Cafestol levels of Turkish/Greek coffee prepared in our laboratory were lower than field samples. This may be due to a more careful decantation of laboratory made brews. In Turkish/Greek coffee brew as consumed, 75– 90% of the diterpenes were carried by floating coffee fines (data not shown). Since cafestol and kahweol from such coffee grounds raise serum cholesterol (Urgert et al., 1995), the amount of coffee fines in Middle Eastern coffee brews will largely determine their hyperlipidemic effect. Cross-sectional studies in Israel, where coffee is mostly brewed by boiling or incubating powdery grounds, have indeed shown higher cholesterol levels in coffee consumers (Kark et al., 1985; Kark, 1990; Green and Harari, 1992), but to our knowledge this has not yet been confirmed experimentally.

Other Coffee Types. French press coffee, also known as plunger or cafetiere coffee, is becoming popular in North America, northern Europe, and Australia (IARC Working Group, 1991). It provided 3-5mg of cafestol per cup, which is similar to levels in Scandinavian boiled coffee and Turkish/Greek coffee. Therefore, French press coffee will raise cholesterol if drunk in large quantities (Figure 6), and people at elevated risk of coronary heart disease should be advised not to drink more than a few cups of French press coffee per day.

In our study, cafestol concentrations per 100 mL were highest in espresso coffee, but since espresso is consumed in small servings (Petracco, 1989), cafestol content per cup was only 1-2 mg. It thus remained well below those of Scandinavian, Turkish/Greek, and French press coffee. In Italy, most of the coffee consumed is mocha coffee (Figure 1) (IARC Working Group, 1991), which contained about 1 mg of cafestol per cup. Cross-sectional data from Italy indicated higher serum lipid levels in coffee drinkers (Panico et al., 1990; Salvaggio et al., 1991; D'Avanzo et al., 1993), but experimental studies have not confirmed a cholesterolelevating effect of mocha or espresso (Finocchiaro et al., 1990; Scaccini and D'Amicis, 1993).

Espresso samples from other countries contained less cafestol despite larger serving sizes (Table 1). This might be attributable to differences in brewing strength (IARC Working Group, 1991), which in the present study was a major determinant of cafestol content. Diterpene levels also varied slightly with espresso device and with roasting grade of the beans used (data not shown). Other factors that might influence lipid levels are steam pressure, contact time of steam with grounds, and mesh width of the filter grid. However, at an average cafestol content of 1 mg per cup of espresso or mocha coffee, consumption of 5 cups/day will raise cholesterol by only 2.5 mg/dL (0.06 mmol/L). Thus, moderate intakes of espresso will have negligible effects on serum cholesterol and coronary heart disease risk.

Pouring boiling water by hand on grounds in a goldplated permanent filter resulted in 2.5 mg of cafestol per cup; possibly the grounds are swirled up so that smaller coffee particles pass through the filter. When we applied permanent gold or nylon filters in an electric drip filter coffee maker, resulting diterpene levels were negligible, as were those for all paper-filtered brews.

The low levels in percolated coffee were surprising. Possibly the bed of coffee grounds (Figure 1) acts as a filter that retains cafestol and kahweol. Prior to the advent of drip filters in the 1960s, percolators were the major type of coffee makers used in the United States. Our data suggest that percolated coffee does not raise serum cholesterol and that changes in coffee brewing practices thus have had little effect on coronary heart disease risk in the United States.

Instant (Soluble) Coffees and Coffee Grounds. Predicted effects of consumption of instant coffee on serum lipids through its cafestol content are minimal (Figure 6), which is in line with results of clinical trials (Aro et al., 1985; Burr et al., 1989).

Cafestol levels in commercial ground coffees varied little and were unaffected by decaffeination. Our measurements thus provide no support for a presumed relation of decaffeinated coffee with raised serum cholesterol (Superko et al., 1991) or with higher risk of cardiovascular disease (Grobbee et al., 1990).

Coffee grounds with low levels of cafestol were blends containing Robusta beans, as was indicated by concurrent higher levels of 16-O-methylcafestol (Speer and Mischnick, 1989) and lower levels of kahweol (Viani, 1988). Higher proportions of Robusta beans in commercial coffee blends would cause lower intake levels of coffee diterpenes. Consumers in most European countries and in the United States prefer Arabica beans, and the contribution of Robusta beans to blends is usually minimized in those countries (Debry, 1994).

Roasting has been reported to eliminate cafestol and kahweol (Nackunstz and Maier, 1987), but in darkroasted Arabica beans—which had lost 26.5% of their initial mass—we found no decrease in diterpene concentration (Figure 5). Although our roasting procedure might have been slightly different from industrial procedures—commercial roasts range from 13% to 22% roasting loss (Viani, 1986)—there appears to be only little effect of roasting on diterpenes in commercial grounds.

Conclusions. Our results predict that chronic consumption of 5 or more cups of French press coffee or Turkish/Greek coffee per day could increase serum cholesterol and thus coronary risk similar to that reported previously for Scandinavian boiled coffee. For espresso and mocha coffee, consumption of 15 or more cups per day is required for the same effect. Effects of instant, filtered, and percolated brews are negligible.

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LITERATURE CITED

- Ahola, I.; Jauhiainen, M.; Aro, A. The hypercholesterolaemic factor in boiled coffee is retained by a paper filter. J. Intern. Med. 1991, 230, 293-297.
- Aro, A. The effect of coffee on serum lipids and its clinical considerations. Cardiovasc. Risk Factors 1993, 3, 238-243.
- Aro, A.; Kostiainen, E.; Huttunen, J. K.; Seppälä, E.; Vapaatalo, H. Effects of coffee and tea on lipoproteins and prostanoids. *Atherosclerosis* 1985, 57, 123-128.
- Burr, M. L.; Gallacher, J. E. J.; Butland, B. K.; Bolton, C. H.; Downs, L. G. Coffee, blood pressure and plasma lipids: a randomized controlled trial. *Eur. J. Clin. Nutr.* 1989, 43, 477-483.
- D'Avanzo, B.; Santoro, L.; Nobili, A.; La Vecchia, C.; GISSI-EFRIM Study Group. Coffee consumption and serum cholesterol. *Prev. Med.* **1993**, *22*, 219-224.
- Debry, G. Coffee and Health; John Libbey Eurotext: London, 1994.
- Finocchiaro, C.; Pezzana, A.; Pernigotti, L.; Bo, M. The influence of coffee on plasma lipids. Coffee and Coronary Heart Disease; Thelle, D. S., van der Stegen, G., Eds.; The Nordic School of Public Health: Göteborg, Sweden, 1990; pp 101-105.
- Fried, R. E.; Levine, D. M.; Kwiterovitch, P. O.; Diamond, E. L.; Wilder, L. B.; Moy, T. F.; Pearson, T. A. The effect of filtered-coffee consumption on plasma lipid levels. JAMA, J. Am. Med. Assoc. 1992, 267, 811-815.
- Green, M. S.; Harari, G. Association of serum lipoproteins and health-related habits with coffee and tea consumption in free-living subjects examined in the Israeli CORDIS study. *Prev. Med.* 1992, 21, 532-545.
- Grobbee, D. E.; Rimm, E. B.; Giovannucci, E.; Colditz, G.; Stampfer, M.; Willett, W. Coffee, caffeine, and cardiovascular disease in men. N. Engl. J. Med. 1990, 323, 1026-1032.
- Heckers, H.; Gobel, U.; Kleppel, U. End of the coffee mystery: diterpene alcohols raise serum low-density lipoprotein cholesterol and triglyceride levels. J. Intern. Med. 1994, 235, 192-193.
- IARC Working Group. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol. 51. Coffee, tea, mate, methylxanthines and methylglyoxal; International Agency for Research on Cancer: Lyon, France, 1991.
- Kark, J. D. Coffee consumption and cholesterol levels in Israel. In Coffee and Coronary Heart Disease; Thelle, D. S., van der Stegen, G., Eds.; The Nordic School of Public Health: Goeteborg, Sweden, 1990; pp 17-23.
- Kark, J. D.; Friedlander, Y.; Kaufmann, N. A.; Stein, Y. Coffee, tea, and plasma cholesterol: the Jerusalem Lipid Research Clinic prevalence study. Br. Med. J. 1985, 291, 699-704.
- Nackunstz, B.; Maier, H. G. Diterpenoids in coffee. III. Cafestol and kahweol. Z. Lebensm. Unters. Forsch. 1987, 184, 494-499.
- Panico, S.; Celentano, E.; Krogh, V. Coffee and blood lipids in Italy. In Coffee and Coronary Heart Disease; Thelle, D. S. van der Stegen, G., Eds.; The Nordic School of Public Health: Göteborg, Sweden, 1990; pp 63-70.
- Petracco, M. Physico-chemical and structural characterisation of "espresso" coffee brew. In *Treizième Colloque Scientifique*

International sur le Café, Paipa, 21–25 août 1989; Association Scientifique Internationale du Café (ASIC): Paris, 1989; pp 246–261.

- Pietinen, P.; Aro, A.; Tuomilehto, J.; Uusitalo, U.; Korhonen, H. Consumption of boiled coffee is correlated with serum cholesterol in Finland. Int. J. Epidemiol. 1990, 19, 586-590.
- Ratnayake, W. M. N.; Hollywood, R.; O'Grady, E.; Stavric, B. Lipid content and composition of coffee brews prepared by different methods. *Food Chem. Toxicol.* **1993**, *31*, 263-269.
- Salvaggio, A.; Periti, M.; Miano, L.; Quaglia, G.; Marzorati, D. Coffee and cholesterol, an Italian study. Am. J. Epidemiol. 1991, 134, 149-156.
- Scaccini, C.; D'Amicis, A. Italian style coffee and serum cholesterol. In Quinzième Colloque Scientifique International sur le Café, Montpellier, 6-11 juin 1993; Association Scientifique Internationale du Café: Paris, 1993; pp 491-495.
- Speer, K.; Mischnick, P. 16-O-methylcafestol-a new diterpene in coffee. Discovery and identification. Z. Lebensm. Unters. Forsch. 1989, 189, 219-222.
- Superko, H. R.; Bortz, W.; Williams, P. T.; Albers, J. J.; Wood, P. D. Caffeinated and decaffeinated coffee effects on plasma lipoprotein cholesterol, apolipoproteins, and lipase activity: a controlled, randomized trial. Am. J. Clin. Nutr. 1991, 54, 599-605.
- Thelle, D. S.; Heyden, S.; Fodor, J. G. Coffee and cholesterol in epidemiological and experimental studies. *Atherosclerosis* 1987, 67, 97–103.
- Urgert, R.; Schulz, A. G. M.; Katan, M. B. Effects of cafestol and kahweol from coffee grounds on serum lipids and serum

liver enzymes in humans. Am. J. Clin. Nutr. **1995**, 61, 149–154.

- Van Dusseldorp, M.; Katan, M. B.; van Vliet, T.; Demacker, P. N. M.; Stalenhoef, A. Cholesterol-raising factor from boiled coffee does not pass a paper filter. Arterioscler. Thromb. 1991, 11, 586-593.
- Viani, R. Coffee. In Ullmann's Encyclopedia of Industrial Chemistry; VCH Verlagsgesellschaft: Weinheim, 1986; pp 315-339.
- Viani, R. Physiologically active substances in coffee. In Coffee. Vol. 3: Physiology; Clarke, R. J., Macrae, R., Eds.; Elsevier Applied Science: London, 1988.
- Weusten-van der Wouw, M. P. M. E.; Katan, M. B.; Viani, R.; Huggett, A. C.; Liardon, R.; Lund-Larsen, P. G.; Thelle, D. S.; Ahola, I.; Aro, A.; Meyboom, S.; Beynen, A. C. Identity of the cholesterol-raising factor from boiled coffee and its effects on liver function enzymes. J. Lipid Res. 1994, 35, 721-733.

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