health effects of unfiltered coffee

diterpenes in coffee and their effects on blood lipids and liver enzymes in man

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Rob Urgert

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Stellingen

1. Cafestol grijpt sterker aan op de cholesterolhuishouding van de mens dan kahweol (*dit proefschrift*).

2. Artsen dienen patiënten met een hoog risico op hart- en vaatziekten het drinken van ongefilterde koffie te ontraden (*dit proefschrift*).

3. In epidemiologisch onderzoek naar het verband tussen koffiedrinken en hart- en vaatziekten is navraag naar het type koffie noodzakelijk (*dit proefschrift*).

4. Cafestol is de krachtigste cholesterolverhogende verbinding die van nature in de voeding voorkomt.

5. Er is onvoldoende reden om aan te nemen dat de oorzaak van de *French paradox* ligt in het drinken van rode wijn.

6. In observationeel onderzoek zal de overtuiging dat er een verband bestaat de kans op het vinden van dat verband aanzienlijk vergroten.

7. Wetenschappelijke tijdschriften zouden onderzoekers moeten toestaan om manuscripten anoniem voor publikatie aan te bieden.

8. Brede toepassing van de techniek om voedselgewassen genetisch te wijzigen zal de verschillen in welvaart tussen Noord en Zuid verder doen toenemen.

9. De houding tegen rokers neemt steeds minder de vorm aan van een houding, en steeds meer die van een hysterie.

10. Veel misverstanden tussen wetenschappers en journalisten zouden voorkomen worden als de eersten zich meer in journalistiek en de laatsten zich meer in wetenschap zouden interesseren.

11. De inburgering van vreemde woorden en uitdrukkingen verrijkt de Nederlandse taal.

12. De in de horeca gebruikte slagzin *De koffie staat klaar!* garandeert geen verse koffie.

13. Vroeger was alleen de toekomst beter.

Stellingen behorend bij het proefschrift
Health effects of unfiltered coffee by Rob Urgert
Wageningen, 4 april 1997

Abstract

Health effects of unfiltered coffee: diterpenes in coffee and their effects on blood lipids and liver enzymes in man

PhD thesis by Rob Urgert, Department of Human Nutrition, Wageningen Agricultural University, Wageningen, the Netherlands, April 4, 1997

Boiled coffee raises blood levels of cholesterol and triglycerides in man. Cafestol and kahweol are responsible. These diterpenes also raise blood levels of alanine aminotransferase (ALT). The objective of the present studies was to further specify the health effects of cafestol and kahweol.

Unfiltered coffee contains up to 5 g of grounds per liter. Intake of 8 g/day of such grounds for 3 weeks raised cholesterol by 0.65 mmol/L and ALT by 18 U/L in healthy volunteers. Diterpenes in floating grounds thus contribute to the hyperlipidaemic and ALT-elevating effects of unfiltered coffee. Chemical analyses showed that boiled, cafetière (also called French press), and Turkish coffee are rich in diterpenes. Levels are moderate in espresso and mocha coffee, and negligible in percolated, instant, and filtered coffee.

We studied the separate activities of cafestol and kahweol in a randomised, double-blind cross-over study with 10 male volunteers. Intake of 63 mg/day of cafestol for 4 weeks raised cholesterol by 17%, triglycerides by 86%, and ALT by 78%. Additional intake of 51 mg/d of kahweol only marginally raised the lipid responses, but more than doubled the ALT responses. Cafestol therefore is more hyperlipidaemic than kahweol, but both affect liver cells.

To study long term effects, we gave 46 volunteers 0.9 liter/day of either filtered or cafetière coffee for 6 months in a randomised experiment. ALT levels were still raised by 45%, and LDL by 9%, after 6 months of intake of cafetière coffee, but most of the initial rise in triglycerides had disappeared.

Lipoprotein(a) is an atherogenic particle made by the liver. We found that lipoprotein(a) levels were 65% higher in chronic drinkers of boiled coffee than in peers drinking filtered coffee. However, supplements rich in diterpenes lowered lipoprotein(a) in four experiments.

In conclusion, the strong and persistent effects on total and LDL cholesterol levels are a good reason to advise patients with a high coronary risk to limit the intake of brews rich in cafestol. Effects of unfiltered coffee on triglycerides and lipoprotein(a) may be insignificant for atherogenic risk. The effects of cafestol and kahweol on liver cells may be innocuous, but coffee drinkers with raised levels of alanine aminotransferase might also do well in abstaining from unfiltered coffee.

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1

general introduction

ABSTRACT

In 1963, the theory arose that coffee drinking could increase the risk of coronary heart disease. The majority of people in Western society drink coffee daily, and coronary heart disease is a common cause of death. As a result, the presumed link between coffee and heart disease has produced a myriad of reports in scientific journals and in the lay media.

Coronary heart disease involves a reduction in blood flow to the heart muscle. Accumulation of fatty deposits in the artery wall -- or atherosclerosis -- is the main cause of reduced blood flow. The risk of atherosclerosis, and thus of coronary heart disease, is increased by many factors. The most notable are cigarette smoking, a high blood pressure, diabetes, and a high concentration of cholesterol in the blood.

Coffee beans contain the diterpenes cafestol and kahweol. They are released by hot water from ground coffee, and are present in brews that are not prepared with a paper filter. Cafestol and kahweol strongly affect lipid metabolism in humans. They also seem to affect liver cells, as they modify the blood level of various enzymes from the liver. This thesis focuses on the health consequences of drinking unfiltered coffee by further specifying the effects of preparations rich in coffee diterpenes on blood levels of lipids and liver enzymes in humans.

This chapter summarizes the history of coffee and the line of research which has resulted in the identification of coffee diterpenes as the cholesterol-raising factors in coffee. An outline of the thesis is given at the end of this chapter.

THE HISTORY OF COFFEE

The first written words about coffee originate from the ninth century when a Persian doctor reported on the digestive properties of coffee. It is believed that coffee was first cultivated in the sixth century. Arab traders and pilgrims took coffee from Ethiopia to Mocha in Yemen, from where it spread throughout the Arabian peninsula. Originally, the coffee beans and cherries were chewed as a stimulant against fatigue, and it was only in the middle of the fifteenth century that coffee became common as a beverage [1]. The habit of drinking coffee rapidly grew throughout the Ottoman empire in the sixteenth century, and in the seventeenth century, coffee crossed the Mediterranean Sea, spread through Europe, and reached the British settlements in North America [2].

The Dutch smuggled a coffee plant out of the port of Mocha in 1690 and succeeded in cultivating coffee on a commercial basis in Java. Halfway through the eighteenth century, the yearly import of coffee in Amsterdam amounted to 1,400 kg of beans. The Dutch supremacy in coffee trading ended when they donated a seedling from a coffee plant to Louis XIV. He planted it in the Jardin des Plantes in Paris and, on royal bequest, made it the ancestor of most of the coffee trees planted during the 18th century in the Western hemisphere [2].

Nowadays, coffee plantations all over the world yield 6 x 10^9 kilograms of beans each year. About three-quarters of the world coffee production consist of *Coffea arabica* (arabica) beans, the remainder being *Coffea canephora* (robusta) beans. Most coffee is consumed in Europe, with an average per capita consumption of 4.6 kg of coffee beans per year [3]. Within Europe, coffee is most popular in Scandinavian countries, Austria, and the Netherlands (figure 1).

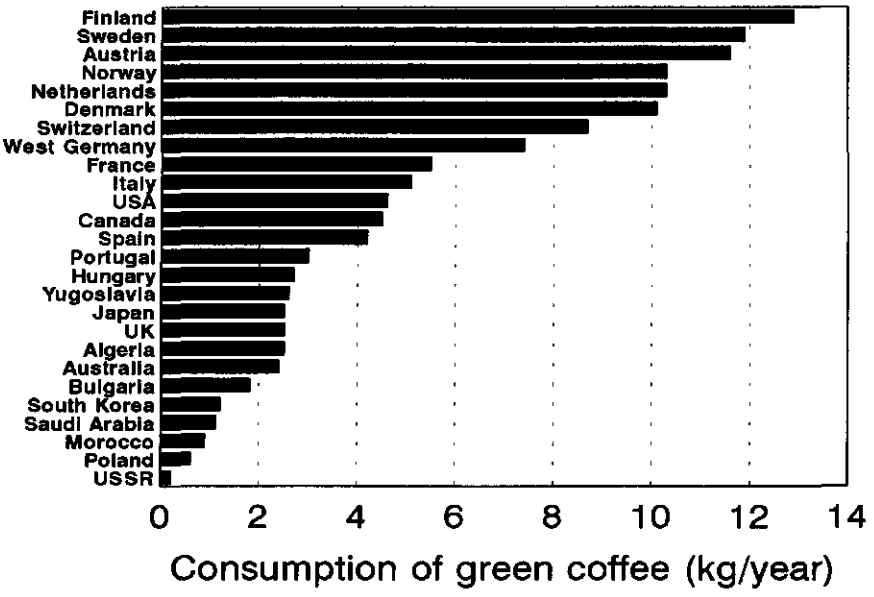

FIGURE 1. *Per capita* consumption of green coffee in selected countries in kilograms per year. Consumption is estimated from data on imports minus exports of all forms of coffee beans adjusted for changes in inventories [3]. Each two kg of green beans per year roughly correspond to one cup of coffee per day.

COFFEE AND CORONARY HEART DISEASE

In 1963, Paul and coworkers suggested that coffee drinking could increase the risk of developing heart disease [4]. Many studies that followed could not confirm this, although some found a link [5,6]. The evidence was insufficient to incriminate caffeine, as controlled studies showed no profound effect of caffeine, either on blood pressure [7,8] or on blood lipid levels [9-12]. Paul's original finding [4] later appeared to be due to confounding by smoking habits [13]. Nevertheless, the incoherent messages brought by scientists kept stirring up people's feelings about an unfavourable effect of coffee on the heart.

In 1983, findings from a cohort of 14,000 Norwegians put things into a new perspective; Thelle and colleagues reported a strong positive relation between coffee drinking and the blood level of cholesterol [14]. Again, such a link was not observed in other Europeans or Americans [15]. A series of both epidemiological surveys [16-20] and experimental studies [21-26] then unravelled the mystery: coffee brewed by boiling grounds with water and consumed unfiltered -- as was common practice in Norway -- strongly raised cholesterol levels, but filtered coffee as consumed in Western European countries and in America did not.

THE CHOLESTEROL-RAISING FACTORS IN COFFEE BEANS

The next question was which particular compound or characteristic of boiled coffee was responsible for its cholesterol-raising effect. A major step in answering this question was the discovery that boiled coffee contains some lipids which are absent in filtered coffee [23,24]. The cholesterol-raising factor was obviously among these lipids; consumption of a lipid-rich fraction from boiled coffee [27] or intake of oils pressed from coffee beans [20,28,29] strongly raised cholesterol and triglyceride levels in volunteers. Two groups of investigators [20,30] then simultaneously reported that the responsible factor(s) belonged to the class of lipids called diterpenes.

The two major coffee diterpenes are cafestol and kahweol (figure 2). They are natural constituents of coffee beans [2], and are released from roast and

ground coffee beans by hot water. Cafestol and kahweol do not pass a paper filter, but, in unfiltered brews, they are present in minuscule oil droplets and in floating grounds. Coffee diterpenes are powerful modulators of lipid metabolism in humans; each twenty milligrams of diterpenes ingested per day for four weeks raises blood levels of total cholesterol by three percent, and triglycerides by ten or more percent [20,30]. Intake of cafestol and kahweol for short periods also modifies the blood levels of various liver enzymes [20,28], which may point to changes in the homeostasis of liver cells [31].

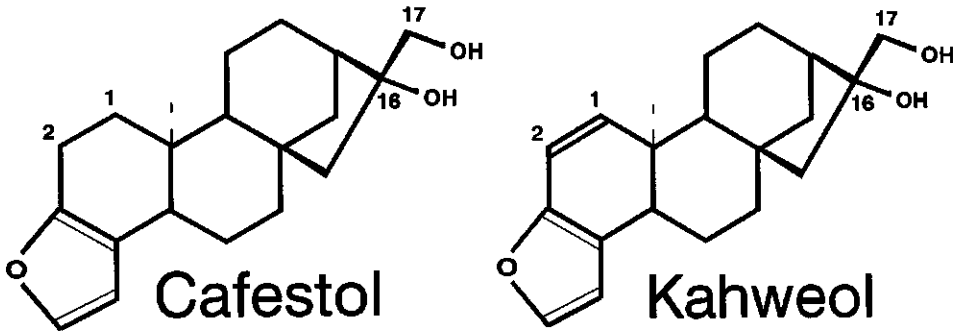

FIGURE 2. Structure of the coffee diterpene alcohols cafestol and kahweol. Compared to cafestol, kahweol has an additional double bond between the C1 and C2 carbon. Diterpenes occur in coffee beans either as free alcohols or esterified to fatty acids at the C17-position [2].

BLOOD LIPIDS OF INTEREST

Cholesterol

The fat-soluble compound cholesterol is transported through the blood in specialised carrier proteins called lipoproteins. Lipoproteins are characterised by their relative density. About 70 percent of total serum cholesterol is carried by low-density lipoproteins (LDL) which play a central role in the delivery of cholesterol from the liver to peripheral tissues [32]. Most of the remainder is carried by high-density lipoproteins (HDL) which are essential in the 'reverse' cholesterol transport, from peripheral tissues back to the liver [33]. Consumption of dietary fatty acids and cholesterol [34], alcohol intake [35], and obesity [36] are important

determinants of the blood level of cholesterol, or of its distribution over different particles.

A high blood level of cholesterol increases the risk of atherosclerotic diseases. Prospective studies have shown that a 10 percent increase in total cholesterol implies a 20 to 50 percent increase in the risk of heart disease [37]. Clinical, epidemiological, and genetic studies convincingly demonstrate that this risk increase is mainly due to the level of LDL cholesterol [32]. In contrast, there is abundant evidence that a high blood level of HDL cholesterol lowers the risk of coronary heart disease [33,38]. As yet, no trials have specifically studied whether a change in HDL cholesterol will result in a change in coronary risk; the benefit of raising HDL cholesterol must still be definitively proven.

Triglycerides

Triglycerides consist of three fatty acids bound to one glycerol molecule. Triglycerides obtained from dietary intake are carried from the intestines to the liver by 'chylomicrons', and are subsequently transported to peripheral tissues by very-low-density lipoproteins (VLDL) [39]. High triglyceride levels are also associated with increased coronary risk [40-42], but it is controversial whether triglyceride level is an independent risk factor for coronary heart disease. For instance, adjustment for HDL cholesterol tends to eliminate the relationship between triglyceride levels and coronary risk [41]. It is also unclear whether levels in the fasting state (when most of the triglycerides are in VLDL) are similarly atherogenic to those in the postprandial state (when most are in chylomicrons) [42]. Therefore, more studies are needed to fully clarify the role of triglycerides in the development of atherosclerosis.

Lipoprotein(a)

Lipoprotein(a) [Lp(a)] is distinguished from LDL only by the presence of a large apolipoprotein [apo(a)] [43]. Despite the structural likeness of Lp(a) and LDL, Lp(a) levels in serum are unaffected by many interventions known to affect levels of LDL cholesterol [44]. Dietary influences on Lp(a) levels are also minimal, except for dietary *trans* fatty acids which cause a modest rise [45-47]. The claims that

fish oils [48,49] and high doses of ascorbic acid [50] lower Lp(a) levels could not be verified [51-55].

An extensive body of laboratory evidence indicates that Lp(a) could be critical in the development of atherothrombotic diseases [56]. Many prospective studies have also shown a higher risk of atherothrombotic disease in individuals with high Lp(a) levels [57-64], but some prospective studies were negative [65-68]. The latter do not necessarily refute an effect of Lp(a) on cardiovascular health, but call for more studies to define the strength of the relationship and its consistency among different populations.

BLOOD LIVER ENZYMES OF INTEREST

Alanine and aspartate aminotransferase

Levels of aminotransferases in blood are employed in clinical practice as indicators of hepatocellular damage. In liver cells, alanine aminotransferase (ALT, SGPT) is solely found in the cytoplasm, whereas aspartate aminotransferase (AST, SGOT) is predominantly found in the mitochondria. The aminotransferases are released from hepatic cells as a consequence of increased membrane permeability as well as membrane breakage. Alanine aminotransferase is the more sensitive and specific test of acute hepatocellular damage, whereas rises in aspartate aminotransferase are more marked in chronic damage [31]. Rises of 50 to 100 times the upper limit of the laboratory reference range are observed with liver diseases or use of hepatotoxic drugs [69]. Smaller increases, often without clinical symptoms, are found in alcoholics and obese people [70,71].

Healthy volunteers who consumed coffee diterpenes for four to six weeks showed raised blood levels of alanine aminotransferase, and, to a lesser extent, of aspartate aminotransferase [20,28]. Blood levels did not exceed the upper limit of normal in most volunteers, and normalised when intake of diterpenes was stopped. These alterations most likely indicate small-scale injury to liver cells [31]. However, chronic consumers of coffee rich in diterpenes did not have elevated levels of aminotransferases [20,72], which suggests that the hepatocellular effect of cafestol and kahweol is transient.

γ-Glutamyltransferase and alkaline phosphatase

Although γ-glutamyltransferase and alkaline phosphatase are found in many organs, their circulating levels are used in clinical practice as indicators of cholestatic disease, i.e. impaired bile flow. Blood levels of γ-glutamyltransferase are also used to monitor alcohol abuse [31].

Levels of γ-glutamyltransferase and alkaline phosphatase in serum were slightly reduced upon intake of coffee diterpenes, whereas a rebound rise in γ-glutamyltransferase was observed upon cessation of treatment [20]. Chronic boiled-coffee drinkers had lower γ-glutamyltransferase levels than filter coffee drinkers [73], which suggests that this effect persists with chronic intake.

OBJECTIVE AND OUTLINE OF THE THESIS

The objective of our research was to further specify the health effects of drinking unfiltered coffee in humans. This objective was subdivided into five prime questions:

Are cafestol and kahweol available from grounds?

Intake of coffee diterpenes dissolved in oils raised cholesterol levels [20,28-30], but their efficacy from floating grounds was unknown; unfiltered coffee brews may contain a fair amount of diterpenes carried by such particles. We measured the amount of floating grounds in coffee brews, and, in two experiments with healthy volunteers, we studied whether consumption of such grounds could affect blood levels of lipids and liver enzymes (Chapter 2).

What are the determinants of the diterpene content of coffee?

We developed a gas chromatography method to analyse diterpene content and applied it to a range of brews collected in countries where a particular type of

coffee is popular. We also prepared a range of brews under standard laboratory conditions to examine factors that influence diterpene content (Chapter 3).

Are cafestol and kahweol both effective?

Kahweol occurs only in the major coffee strain arabica, whereas cafestol occurs in both robusta and arabica beans [2]. Oils pressed from robusta beans raised serum cholesterol [20,28,29], which suggests that cafestol is active. However, the activity of kahweol was unknown. We examined the separate effects of cafestol and kahweol on blood lipids and liver enzymes in a randomised cross-over study with ten healthy male volunteers who received either pure cafestol alone or a mixture of cafestol and kahweol for four weeks (Chapter 4).

Does the hepatocellular effect persist with prolonged intake?

Most of the evidence of the hepatocellular effect of cafestol and kahweol was derived from short-term experiments. As chronic drinkers of boiled coffee did not have higher levels of alanine aminotransferase than matched filter coffee drinkers [20], we hypothesised that the effect is transient with prolonged intake. In addition, the time course of the changes in total and LDL cholesterol as induced by coffee diterpenes was remarkable; a steady state was not reached within four weeks of treatment [20,30]. We studied the effects of prolonged intake of cafestol and kahweol on serum levels of liver enzymes and lipids in a randomised parallel trial with 46 healthy volunteers who received either cafetière or filtered coffee for six months (Chapter 5).

Do cafestol and kahweol affect serum lipoprotein(a)?

The increase in alanine aminotransferase levels with diterpene intake may point to slightly disturbed hepatocyte integrity [31], and the impact on lipid metabolism could also be due to effects on the liver [74]. Apolipoprotein(a) is synthesised in the liver [75], and circulating levels of Lp(a) are largely determined by the rate of production [76]. The hypothesis that cafestol and kahweol affect the synthesis and secretion of apo(a), thereby reducing the circulating level of Lp(a),

was tested in an observational study with Norwegian coffee consumers (Chapter 6) and in four experiments with Dutch volunteers (Chapter 7).

Finally, chapter 8 summarizes the knowledge about the cholesterol-raising factors from coffee beans, including the information brought in by the present studies. It also describes the implications of drinking unfiltered coffee for health. Chapter 9 describes the main conclusions of our studies and recommendations for further research.

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2

effects of cafestol and kahweol from coffee grounds on serum lipids and serum liver enzymes in humans

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/L and French press coffee 1.5 g/L of fines. Intake of 8 g of fine grounds per day for three weeks increased cholesterol by 0.65 mmol/L (95% Confidence Interval, 0.41 to 0.89 mmol/L) and ALT by 18 U/L (95% Confidence Interval, 4 to 32 U/L) relative to controls (n = 7 per group). In a cross-over study (n = 15), mean serum cholesterol was 4.9 mmol/L after consumption of both fine and coarse grounds for 10 days (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 (AMERICAN JOURNAL OF CLINICAL NUTRITION 1995; 61: 149-154).

INTRODUCTION

Scandinavian 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]. Cafestol plus kahweol esters also elevate at least transiently the serum activity of alanine aminotransferase (ALT) in serum and depress serum levels of creatinine in humans not accustomed to drinking boiled coffee [5]. The lower serum values of gamma-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 ("plunger" or "cafetiere") coffee and espresso coffee contain diterpenes both in 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 which degree cafestol and kahweol are absorbed from grounds.

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

MATERIALS, SUBJECTS AND METHODS

Particle content of coffee brews

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

Scandinavian boiled coffee was brewed with 10 min boiling, and 5 min settling time. French press coffee (also known as "plunger" or "cafetiere" coffee) was prepared by pouring boiling water onto grounds in a glass jug of 1 liter (Bodum AG, Triengen, Switzerland), and pushing down the metal screen strainer (plunger) after 5 min incubation. Percolated coffee was prepared by recirculating boiling water for 20 min using a household percolator. Espresso coffee was prepared with a household espresso machine (Espresso Duo, Philips, Eindhoven, The 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 minutes. 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, The Netherlands) with a paper (Melitta, Gorinchem, The Netherlands), cotton (Bean Bag, USA), nylon (Prestige, France) or gold filter (Swissgold, Elfo Ag Sachseln, Sachseln, Switzerland). All brews were prepared in fourfold.

Ten mL of each unfiltered brew, espresso, mocha or French press brew was centrifuged at 3000 rpm for 5 min (Centaur 2, MSE, England) and the lipid-containing upper layer was washed away with 1 mL of hexane. The tube was then dried at 105°C, and weighed. For drip filtered and percolated brews, samples of

200 mL were centrifuged in containers of 250 mL at 19200 x g (Highspeed 18, MSE, Crawley, England). The lipid-containing upper layer was washed away with 25 mL of hexane. The particle precipitate was then collected by filtration over paper (Schleicher & Schuell, Dassel, Germany), dried at 105 °C, and weighed.

Effect of coffee grounds on serum lipids and liver enzymes

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

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 rotation blade (Krups KM75, 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, while grounds passing the 1.0 mm sieve were ground to pass an 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 minutes before being divided into daily portions.

We analysed 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 ethanolic KOH, 5 mol/L, and 5α-cholestane was added as an internal standard. The free diterpene alcohols were extracted with diisopropylether, and analysed as trimethylsilylethers on a Hewlett Packard 5890 series II gas chromatograph (Avondale, PA19311, USA). Authenticity and purity of the peaks were verified on a Hewlett Packard G1019A mass spectrometer.

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 gastro-intestinal, liver or kidney diseases. One woman withdrew from study 2 in the first week because of gastro-intestinal discomfort. All other participants completed study 1 (n = 14) or study 2 (n = 15) successfully. They were mostly young, lean, and non-smoking (table 1). They consumed no or only moderate amounts of alcohol and coffee. They did not take medications affecting serum lipids or liver enzymes, and the levels of these were all within normal limits.

TABLE 1. Baseline characteristics of the participants[a].

	Study 1	Study 2
Sex (M/F)	6/8	9/6
Age (years)	24 ± 3	26 ± 5
Body Mass Index (kg/m^2)[b]	21.8 ± 2.1	22.0 ± 2.3
Oral contraceptive users	2	2
Smokers	2	3
Daily coffee consumption (cups)	3 ± 2	3 ± 2
Weekly alcohol consumption (glasses)	7 ± 7	4 ± 3

[a] Values are numbers, or means ± SD

[b] Body weights were measured without shoes or heavy clothing.

Hypotheses and designs

STUDY 1. The hypothesis to be tested was that daily ingestion of eight g of coffee grounds elevates serum cholesterol and ALT activity. Calculations showed that we would need 6 subjects per group to detect a difference in serum cholesterol of 0.50 mmol/L with a power of 90%, and 7 to detect a difference in ALT of 20 U/L ($\alpha = 0.05$).

During the run-in period of 14 days, subjects consumed 125 mL of 'hopjes-caramelvla', a commercially available, sweet-flavoured dairy dessert providing 3.8 g of fat (2.5 g saturated, 1.3 g mono-unsaturated fatty acids) and 12.5 mg of

cholesterol per day. Venous blood samples were taken from each subject on days -3 and 0.

During the test period of 21 days both groups consumed 125 mL of hopjes-caramelvla per day. 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 half an hour 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 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 days after the experiment (day 80).

STUDY 2. We hypothesised 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% ($\alpha = 0.05$).

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

Blood assays

Blood samples were taken after an overnight fast. Sera were obtained by centrifugation, stored at -80°C and analysed within one run. Sera obtained post-

experimentally were analysed separately. Sera were analysed enzymatically for total cholesterol [14] and triglycerides [15]. Mean bias for control sera provided by the Centers for Disease Control (Atlanta, USA) was -2% for total cholesterol and 4% for triglycerides. The coefficient of variation within runs ranged from 0.7 to 2.1%. Creatinine was measured with a modified Jaffé method using a Spectrum kit (Abbott Laboratories, North Chicago, USA) [16]. Serum activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) [17], gamma-glutamyltransferase (γ-GT) [18] and alkaline phosphatase [19] were measured at 37°C using Abbott Spectrum reagents. 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%. Upper limits of normal were 53.5 U/L for ALT activity, 39.7 U/L for AST activity, and 92 U/L for alkaline phosphatase activity, and for γ-GT activity 63 U/L for men and 35 U/L for women.

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 compared using an unpaired, one-tailed t test.

STUDY 2. Effects of particle size were analysed by comparing values obtained on days 11 and 25, i.e. after ten days of consumption of either type of grounds; t tests [20] showed period effects for all serum variables ($p < 0.05$) except triglycerides. However, t tests for equality of carry-over effects from the first into the second period [20] showed similar carry-over 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 zero 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 weight per liter of Scandinavian coffee to about 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. Levels of coffee bean particles (mean ± SD) in various coffee brews as consumed.

Coffee type	Brewing strength	Grind	Particle content
Unfiltered	g/L water		g dry weight/L
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

Effect of coffee grounds on serum lipids and liver enzymes

The 29 out 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 of the 147 daily portions of grounds had been consumed. Dry weights of the grounds portions were (mean ± SD) 7.8 ± 0.2 g/d. The grounds provided 39 ± 4 mg of cafestol and 49 ± 5 mg of kahweol per day (n = 5).

After 21 days of grounds consumption, participants showed a mean rise in serum cholesterol of 0.65 mmol/L (95% Confidence Interval, 0.41 to 0.89 mmol/L) relative to the control group (table 3, figure 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 controls was 18 U/L (95% CI, 4 to 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 ($p < 0.10$). All serum values returned to baseline level after cessation of the treatment.

STUDY 2. Diaries showed that only one out 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 of cafestol and 54 mg of kahweol and the fine grounds 48 mg of cafestol and 56 mg of kahweol per day (n = 1).

Serum cholesterol levels showed a similar response for both treatment sequences (figure 2). Mean levels were 4.9 mmol/L both after consumption of fine and of coarse grounds (table 4). Mean ALT activity in serum was 29 U/L on fine and 21 U/L on coarse grounds (p for difference 0.02). Serum values of triglycerides and AST were higher, and those of γ-GT lower on fine than on course grounds ($p < 0.05$).

TABLE 3. STUDY 1. Mean changes (± SD) in values of serum lipids, liver enzymes, and creatinine in healthy volunteers consuming hopjes-caramelvla (control group) or hopjes-caramelvla with 7.8 g/day of dry weight of fine spent coffee grounds (treatment group) for 21 days, and differences between changes [1].

	Control group (n = 7)	Treatment group (n = 7)	Difference between groups (95% CI)	P-value
Cholesterol (mmol/L)	0.01 ± 0.23	0.66 ± 0.24	0.65 (0.41 to 0.89)	<0.001
Triglycerides (mmol/L)	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
AP (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] To convert cholesterol values to mg/dL multiply by 38.67, triglycerides by 88.54.

TABLE 4. STUDY 2. mean (± SD) values of serum lipids, liver enzymes, and creatinine of 15 healthy volunteers upon daily consumption of hopjes-caramelvla with 6.6 g of fine, and with 7.1 g of coarse grounds for 10 days each in a cross-over design [1].

	Value on fine grounds	Value on coarse grounds	Difference fine-coarse	P-value
Cholesterol (mmol/L)	4.89 ± 0.73	4.86 ± 0.65	0.03 ± 0.71	0.43
Triglycerides (mmol/L)	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
AP (U/L)	63 ± 18	63 ± 15	-0 ± 6	0.41
Creatinine (μmol/L)	86 ± 13	85 ± 13	0 ± 8	0.42

[1] To convert cholesterol values to mg/dL multiply by 38.67, triglycerides by 88.54.

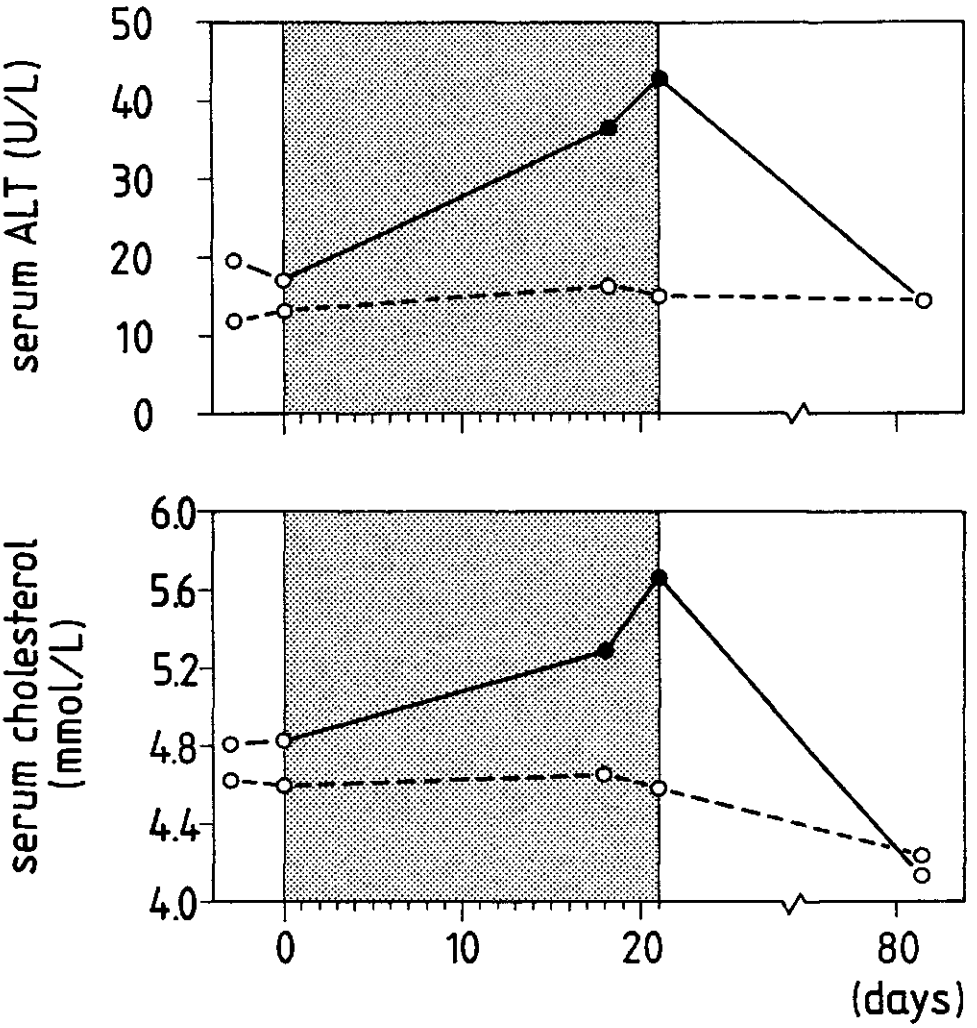

FIGURE 1. STUDY 1. **Mean levels of serum cholesterol and alanine aminotransferase in two groups of 7 healthy volunteers at baseline (○), after daily consumption of 125 mL of hopjes-caramelvla with (●) or without (○) 7.8 g of fine spent coffee grounds, and 59 days after the experiment (○). The grey area indicates the period during which grounds were administered.**

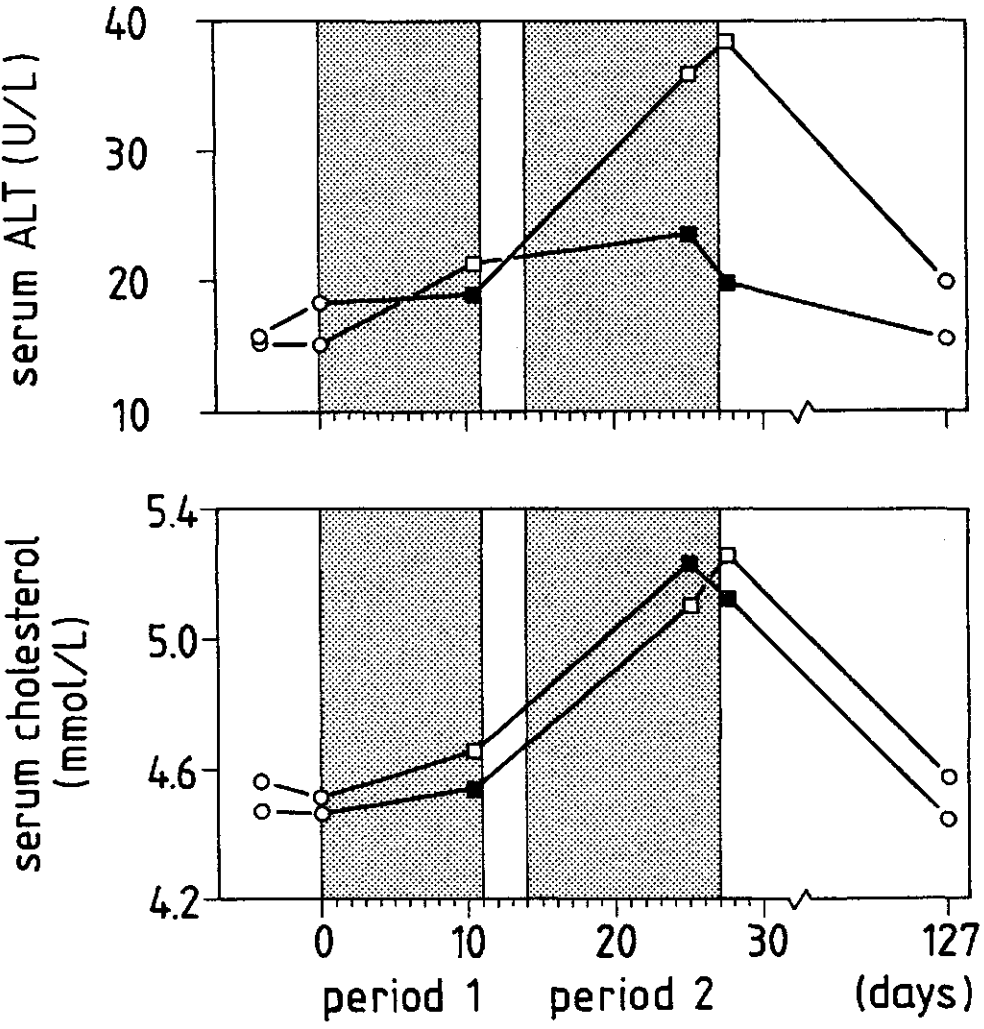

FIGURE 2. STUDY 2. Mean levels of serum cholesterol and alanine aminotransferase in 15 healthy volunteers at baseline (○), after daily consumption of 6.6 g of fine (□) or 7.1 g of coarse grounds (■), and 99 days after the experiment (○) (n = 7 or 8 per group). The grey areas indicate the periods during which grounds were administered.

DISCUSSION

Particle content of coffee brews

Scandinavian boiled coffee, the coffee type most commonly associated with raised cholesterol levels [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 of fines), then the grounds in boiled coffee would provide 11 mg cafestol and 13 mg kahweol per liter of brew. This is 22% of the total amount of 108 mg of diterpenes per liter [8]. Levels for Middle Eastern brews, known variously as "Turkish", "Greek", "Arab" or "Israeli mud", were even higher. Middle Eastern boiled coffee contained 5.3 g/L of fines, which would provide 27 mg of cafestol and 33 mg of kahweol, accounting for about half of 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. This underlines the importance of an analytical method which includes the contribution of ingested coffee fines to the total amount of diterpenes in the brew.

Effect of coffee grounds on serum lipids and enzymes

STUDY 1. In this parallel study, daily ingestion of 8 g of coffee grounds significantly raised serum cholesterol and ALT levels compared to controls. Elevations in these parameters 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 of cafestol and 49 mg of kahweol with coffee grounds resulted in a rise in serum total cholesterol of 0.65 mmol/L (26 mg/dL). We have earlier estimated that each extra 2 mg of 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 were 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 levels rose on average by 1 U/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 to 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 three weeks. In previous studies [5,9], the effects of cafestol and kahweol on serum cholesterol and ALT did not stabilise within four weeks of administration. Thus, some underestimation due to treatment duration may be present.

STUDY 2. The effect of particle size on the availability of cafestol and kahweol was examined in a cross-over study. Comparison of the two particle sizes, however, was complicated by the unexpectedly different cafestol content of the samples, 48 mg of cafestol in fine versus 37 mg in coarse grounds. Later experiments (unpublished) 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 activity and triglycerides could therefore be due to their higher cafestol content, rather than to particle size. However, despite the difference in cafestol, responses of serum cholesterol were highly similar for both treatments (figure 2). Possibly, the effect on serum cholesterol can be induced by lower levels of cafestol than the effect on serum ALT. Furthermore, kahweol could have lessened the difference between the two groups in this study, since 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 parameters 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.

Conclusions

Cafestol and kahweol from coffee grounds raise serum cholesterol and ALT activity similar to cafestol and kahweol from boiled coffee or coffee oil. Since 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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3

levels of the cholesterol-elevating diterpenes cafestol and kahweol in various coffee brews

ABSTRACT

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). We examined diterpene levels in various coffee brews. Scandinavian boiled coffee contained (mean ± SD) 3.0 ± 2.8 mg, French press (also called 'cafetière') coffee 3.5 ± 1.2 mg, and Turkish/Greek coffee 3.9 ± 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 ± 1.0 mg of cafestol per cup, five cups theoretically raising cholesterol by 4 mg/dL. Brewing time had little effect on 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 coffee 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 (JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY 1995; 43: 2167-72).

INTRODUCTION

Scandinavian-type boiled coffee raises serum cholesterol in man [1,2]. The diterpenes cafestol and, possibly, kahweol are responsible for this effect [3,4]. Additionally, cafestol and kahweol appear to affect liver cell metabolism; they increased the activity of alanine aminotransferase and depressed that of gamma-glutamyltransferase in serum. In controlled trials, serum cholesterol rose by about 1 mg/dL (0.03 mmol/L) and alanine aminotransferase by about 1 U/L for each extra 2 mg of cafestol ingested [4]. Levels of cafestol and kahweol in various coffee brews could be used to predict their capacity to affect lipoprotein and liver cell metabolism 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) [5]. Robusta beans are almost devoid of kahweol [6] but contain a third diterpene -- 16-*O*-methylcafestol -

- which is absent in Arabica beans [7]. 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. The oil is retained by a paper filter [8], which explains why paper-filtered coffee shows no [9,10] or only little [11] effect on serum cholesterol. With espresso and mocha coffee, and with French press coffee, also known as cafetière coffee, lipids readily pass the metal filter [8] and the hypercholesterolaemic 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 [5].

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

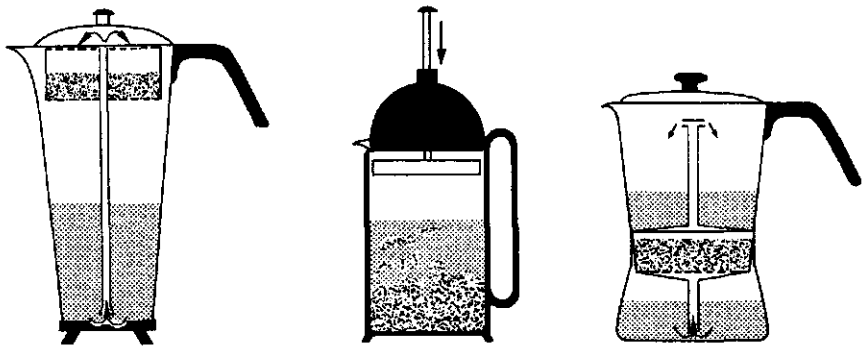

FIGURE 1. Brewing principles for percolated, French press (cafetière), and mocha coffees.

METHODS

Collection of field samples

Boiled coffee was collected from regular consumers in Norway and Finland (n = 14); Turkish/Greek coffee from Turkish and Greek restaurants operated by immigrants in the Netherlands (n = 7) and from retail outlets in Greece and Egypt (n = 4); espresso from bars and restaurants in Italy (n = 10), Spain (n = 2), Switzer-

land (n = 4) and the Netherlands (n = 15); and French press coffee (figure 1) 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.

Thirteen regular and 6 decaffeinated instant (soluble) coffees were analysed. Instant brands included Folgers red and green, Tasters Choice red and green, Sanka To Go decaffeinated (decaf) and Maxwell House (USA), 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 analysed. Brands included Hills Bros red and green, Folgers Mountain Grown regular and decaf, Maxwell House Master Blend and Maxwell House decaf (USA), Albert Heijn Perla, Van Nelle Supra, Cafe Idee, Douwe Egberts Kleintje Koffie and decaf, Douwe Egberts Piazza Espresso, Douwe Egberts roodmerk (The Netherlands), Jacobs Espresso Mastro Lorenzo regular 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:

0% between 0.6 and 1.0 mm, and 25% between 0.42
rounds: 17% larger than 0.6 mm, 26% between 0.42
n 0.2 and 0.42 mm and 9% between 0.075 and 0.2
e obtained by pulverizing fine grounds in a beaker with
5, Solingen, Germany). Ten percent of the powdery
than 0.42 mm, 38% between 0.2 mm and 0.42 mm,
nd 0.2 mm, and 18% smaller than 0.075 mm.
d in triplicate and stored at -20 °C for a maximum of 3

. "Scandinavian" boiled coffee was brewed in the
sely ground beans with water, followed by 5 min of
as decanted until particles of the sediment started to

ypes of Middle Eastern coffee. "Turkish/Greek" coffee
powdery grounds to a foamy boil with water in a
pot. Then the brew was decanted into a cup. "Israeli
ared by pouring boiling water onto powdery grounds in
es, the contents of each cup were decanted again after
o as to mimic usual consumption.

French press coffee (figure 1) was brewed by pouring
ground beans in a glass plunger pot of 1 L (Bodum AG,
pushing down the metal screen strainer (plunger) after

brewed with fine grind. We compared espresso brewed
o machines for household use from Philips (Eindhoven,
from Krups (Solingen, Germany), using three extraction
00 mL), and three roasting grades (light, medium, and

brewed with fine grind in an aluminum mocha-maker
, Italy) (figure 1).
was prepared by recirculating boiling water for 20 min
sing 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, USA), 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 millilitres 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 μL of absolute ethanol (Merck, no. 983). One millilitre 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 μm sieve (Retsch, Haan, Germany). Then, 100-200 mg of grounds was combined with 1 mL of 5α-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 millilitre of demineralised water was added, and the water phase was extracted three times with 2 mL of diisopropyl ether. Three millilitres of demineralised water were 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 μL 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 μL was injected splitless into a Hewlett-Packard 5890 Series II gas chromatograph (Avondale, PA, USA) equipped with a 25 m x 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 period for cafestol, and 2.9% and 5.2%, respectively, for kahweol. Recoveries of cafestol and kahweol (mean ± SD) were 102.2 ± 2.3% and 100.3 ± 2.5%, respectively, if added in the form of coffee oil to paper-filtered coffee (n = 6), and 100.1 ± 3.4% and 99.3 ± 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.

TABLE 1. Mean levels ± SD (ranges) of cafestol, kahweol, and 16-*O*-methylcafestol in coffees collected from bars and restaurants and from regular consumers in a range of countries (cf Methods section). Values are given in terms of mg of free diterpene alcohols per cup. For brews collected from 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.

Type of coffee	Cafestol	Kahweol	16-*O*-Methyl-cafestol
		mg per cup	
Scandinavian boiled (n = 14)	3.0 ± 2.8 (0.8 to 12.1)	3.9 ± 3.4 (1.1 to 14.6)	0.0 ± 0.0 (0.0 to 0.1)
Turkish/Greek (n = 11)	3.9 ± 3.2 (0.5 to 10.0)	3.9 ± 3.9 (0.1 to 10.7)	0.5 ± 0.6 (0.0 to 1.4)
French press (n = 5)	3.5 ± 1.2 (2.3 to 5.5)	4.4 ± 2.1 (2.6 to 8.0)	0.1 ± 0.1 (0 to 0.2)
Espresso			
Italy (n = 10)	1.5 ± 1.0 (0.2 to 2.9)	1.8 ± 1.3 (0.2 to 3.9)	0.1 ± 0.1 (0.0 to 0.3)
Other countries (n = 21)	1.2 ± 0.9 (0.0 to 3.1)	1.4 ± 1.1 (0.0 to 3.9)	0.1 ± 0.1 (0.0 to 0.3)

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 - 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).

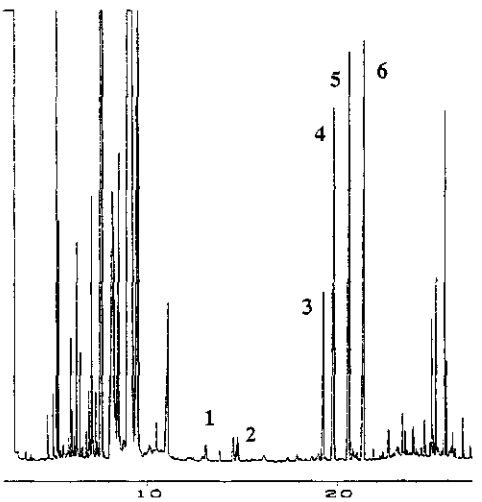

FIGURE 2. Gas chromatogram of silyl derivatives of coffee diterpenes in a Turkish/Greek coffee sample. Peaks: decomposition products of cafestol and kahweol (1, 2), 16-*O*-methylcafestol (3), kahweol (4), cafestol (5), and 5α-cholestane (6). Other peaks represent mostly free fatty acids and phytosterols. For further details, see Methods section.

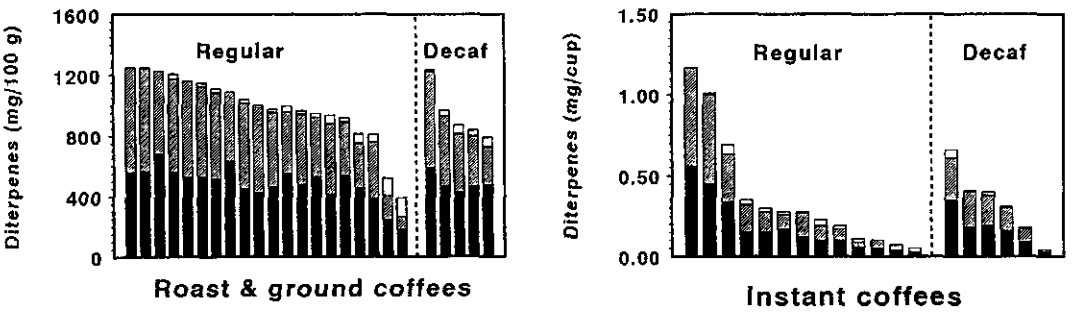

FIGURE 3. Levels of cafestol (solid bar), kahweol (slashed bar) and 16-*O*-methylcafestol (open bar) in instant (soluble) coffee granules and in commercial roast and ground coffees. For instant coffees, values are given as milligrams of free alcohols per 2 g of soluble granules, which is the amount that goes into one cup [5]. For coffee grounds, values are milligrams per 100 g. 'Regular' refers to caffeine-containing products, 'decaf' refers to decaffeinated products.

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.

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%.

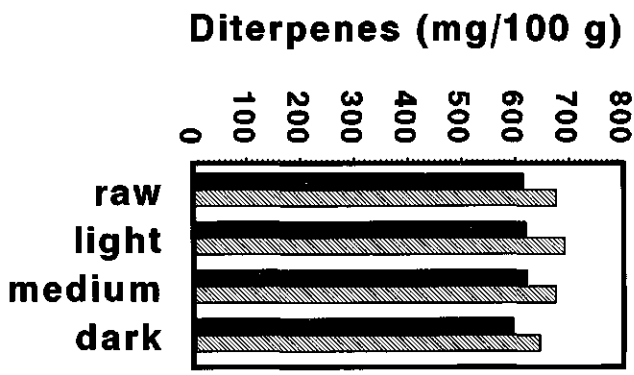

FIGURE 4. Effect of roasting on levels of cafestol (solid bar) and kahweol (slashed bar) in Mexican Arabica beans, expressed as milligrams per 100 g of roasted product. Corresponding weight losses were 24.5% for light, 26.0% for medium, and 26.5% for dark roasted beans.

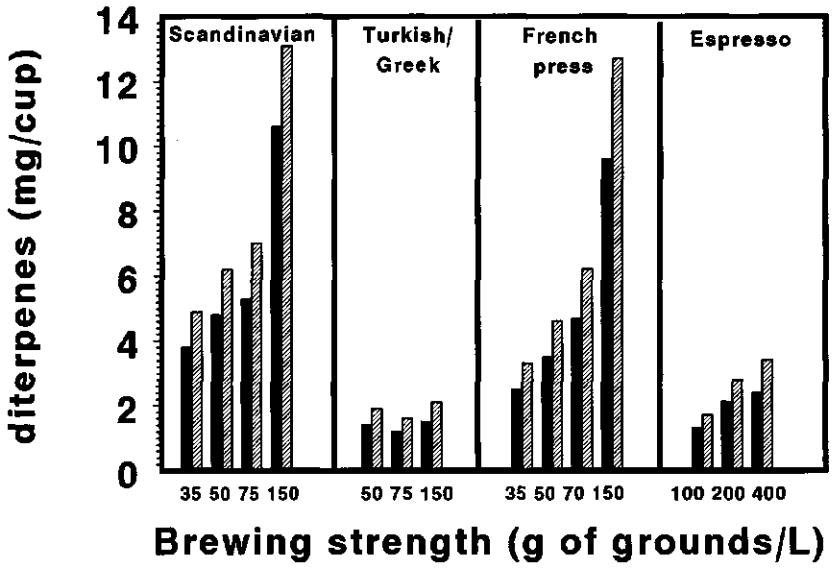

FIGURE 5. Influence of brewing strength (concentration of coffee grounds per liter of fresh water used for brewing) on levels of cafestol (solid bar) and kahweol (slashed bar) in *Scandinavian boiled, Turkish/Greek, French press,* and *espresso coffee prepared* in the laboratory under standard conditions. Brews were prepared in triplicate. Values are given as milligrams of free alcohols per cup of 150 mL (for espresso: 25 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 4%.

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 1.1 ± 0.1 mg of cafestol and 1.4 ± 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 resulted in 2.5 ± 1.2 mg of cafestol per cup for gold-plated (n = 21), 0.8 ± 0.1 mg for nylon (n=3), and 0.1 ± 0.0 mg for paper filters (n=3).

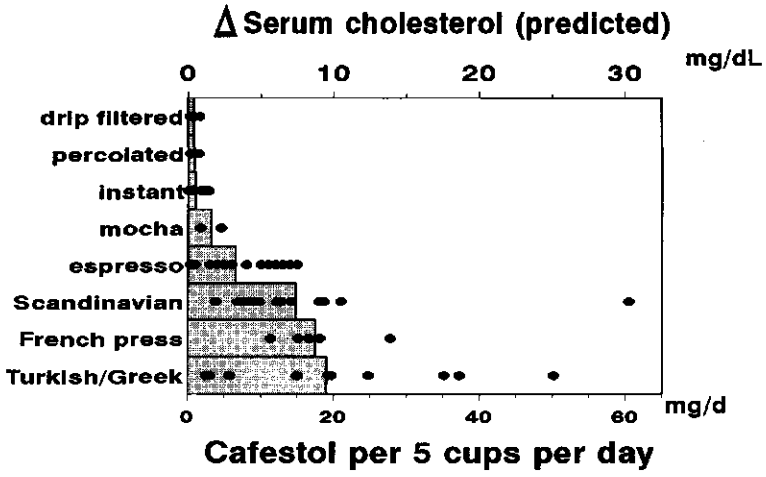

FIGURE 6. Predicted effect on serum cholesterol of daily consumption of 5 cups of various types of coffee. Black dots represent individual and bars mean values of field samples. Estimations of the rise in serum cholesterol are based on the observation of Weusten-van der Wouw et al [4] that every 10 mg of cafestol consumed per day raises serum cholesterol by 5 mg/dL (0.13 mmol/L).

DISCUSSION

The major finding from this study is that Turkish/Greek and French press, also known as plunger or cafetière 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 [4] (figure 6). Finnish 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 [13], and Norwegians consuming 5 or more cups had levels 12 mg/dL higher [4]. 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 [14], the amount of coffee fines in Middle Eastern coffee brews will largely determine their hyperlipidaemic 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 [12, 15, 16], but to our knowledge this has not yet been confirmed experimentally.

Other coffee types

French press coffee, also known as plunger or cafetière coffee, is becoming popular in North America, northern Europe, and Australia [5]. It provided 3-5 mg 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 [17], 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) [5], which contained about 1 mg of cafestol per cup. Cross-sectional data from Italy indicated higher serum lipid levels in coffee drinkers [18, 19, 20], but experimental studies have not confirmed a cholesterol-elevating effect of mocha or espresso [21,22].

Espresso samples from other countries contained less cafestol despite larger

serving sizes (table 1). This might be attributable to differences in brewing strength [5], 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 per 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 gold-plated 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 [23,24].

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 [25] or with a higher risk of cardiovascular disease [26].

Coffee grounds with low levels of cafestol were blends containing Robusta beans, as was indicated by concurrent higher levels of 16-*O*-methylcafestol [7] and lower levels of kahweol [6]. 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 of America prefer Arabica beans, and the contribution of Robusta beans to blends is usually minimised in those countries [27].

Roasting has been reported to eliminate cafestol and kahweol [28], but in dark-roasted 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 [6] -- 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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4

separate effects of the coffee diterpenes cafestol and kahweol on serum lipids and liver aminotransferases

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 and triacylglycerols, and of 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 randomised, double-blind cross-over study, we gave ten healthy male volunteers either pure cafestol (61 – 64 mg/day) or a mixture of cafestol (60 mg/day) and kahweol (48 – 54 mg/day) for 28 days. Relative to baseline values, cafestol raised mean (± SEM) total serum cholesterol by 0.79 ± 0.14 mmol/L (31 ± 5 mg/dL), low-density lipoprotein (LDL) cholesterol by 0.57 ± 0.13 mmol/L (22 ± 5 mg/dL), fasting triacylglycerols by 0.65 ± 0.12 mmol/L (58 ± 11 mg/dL), and alanine aminotransferase by 18 ± 2 U/L (all $P<0.01$). Relative to cafestol alone, the mixture of cafestol plus kahweol increased total cholesterol by another 0.23 ± 0.16 mmol/L (9 ± 6 mg/dL) ($P=0.08$), LDL cholesterol by 0.23 ± 0.16 mmol/L (9 ± 6 mg/dL) ($P=0.09$), triacylglycerols by 0.09 ± 0.10 mmol/L (8 ± 9 mg/dL) ($P=0.20$), and alanine aminotransferase by 35 ± 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 hyperlipidaemic potential of unfiltered coffee mainly depends on its cafestol content. Both cafestol and kahweol raised alanine aminotransferase concentrations, and their hyperlipidaemic effect thus seems not to be coupled with their effect on liver cells (AMERICAN JOURNAL OF CLINICAL NUTRITION 1997; 65: 519-524).

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 to 2 g of lipids per liter, of which about 10 percent are diterpenes [8]. In a series of controlled experiments [9,10], we showed that these diterpenes are responsible for the cholesterol-raising effects of unfiltered coffee. The relation appeared to be linear up to doses of 200 mg of diterpenes per day [10], the amount present in 20 to 30 cups of Scandinavian boiled or French press (also called cafetière) coffee [11].

The mode of action of coffee diterpenes is largely unknown. They raised serum concentrations of alanine aminotransferase (ALT, formerly called SGPT), and

reduced those of γ – glutamyltransferase in humans [10,12]. These alterations may point to changes in the integrity of the liver cell [13]. In addition, coffee diterpenes reduced circulating lipoprotein(a) levels (see chapter 7), which may also be related to changes in liver cell metabolism [14]. We therefore suggested that coffee diterpenes influence lipoprotein metabolism via effects on the liver.

The major coffee diterpenes are *cafestol* and *kahweol* (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 [15,16], indicating that cafestol has hyperlipidaemic potential. However, in the United States of America and in western Europe, arabica beans are preferred over robusta beans [17]. As a result, levels 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 cross – over trial with healthy volunteers.

MATERIALS, 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. Diterpenes naturally occur as fatty acids 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 non – diterpene 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 Pd – Pb – $CaCo_3$ as a catalyst. The diterpene alcohols were re – esterified 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 plus palm oil, w/w 3/2. Red palm oil was used so as to mask the yellowish colour of kahweol. ß – carotene in the supplements provided less than two percent of the recommended daily intake of vitamin A for Dutch adult men [19]. Diterpene contents of the final capsules were assayed as described [10]. Purities of the diterpenes ranged from 92.2 to 99.7 percent; impurities consisted of cafestol and kahweol dipalmitates and free 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 1). Prio approval was obtained from the human ethics committee of our department, and from the Nijmegen University Hospital Ethical Committee.

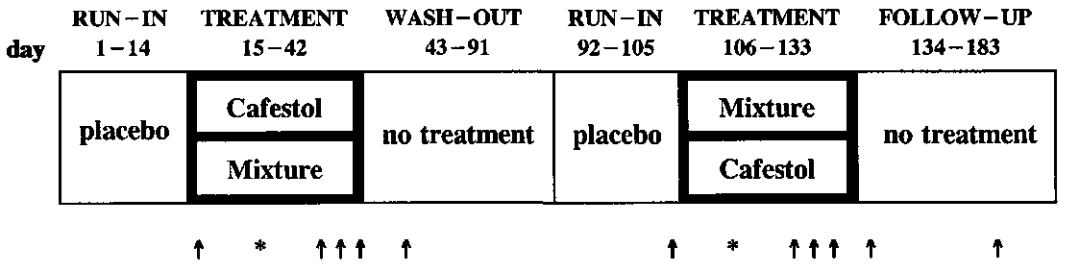

FIGURE 1. Experimental design. 'Cafestol' refers to the palmitate esters of cafestol alone, and 'Mixture' refers to the palmitate esters of cafestol and kahweol. Blood samples (↑) were obtained on days 15 (run – in), 36, 39, 43 (treatment), 57 (wash – out), 106 (run – in), days 127, 130, 134 (treatment), and days 148 and 183 (follow – up). Additional serum samples (*) were obtained to monitor adverse effects during the treatment periods.

We estimated that ingestion of 60 mg of cafestol plus a similar amount of kahweol per day would raise serum cholesterol 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 ten subjects to detect a difference of 0.55 mmol/L with a statistical power of 90%, using a cross – over design ($\alpha = 0.05$).

We recruited ten 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 upon 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 age ($\pm$ SD) was 24 $\pm$ 4 y, and the mean body mass index was 21 $\pm$ 2 kg/m^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 5 placebo capsules providing a total of 2 g of a 3:2 (w:w) mixture of sunflower and palm oil per day. During the first treatment period, five subjects swallowed 5 capsules per day providing 64 mg of cafestol and 1 mg of kahweol (amounts expressed as free alcohols) dissolved in 2 g of the placebo oil mixture. The other five subjects swallowed capsules with 60 mg of cafestol and 54 mg of kahweol, again dissolved in oil. Supplements were switched in the second treatment period; analysed doses of cafestol and kahweol were now 61 and 0 mg for pure cafestol, and 60 and 48 mg for the mixture, respectively. Every two weeks, 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 wash – out and the 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 twenty alcohol – containing beverages per week. 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 (wash – out), 106 (second run – in), 127, 130, 134 (second treatment), and 148 and 183 (follow – up) (figure 1). Additional serum samples were obtained on the 15th day of either treatment period, and were analysed instantly for total cholesterol and alanine aminotransferase 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 more than 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. 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, as described under 'statistics'.

Blood sampling and assays

Serum was obtained by centrifugation, and stored at −80°C. Sera were analysed enzymatically for total [20] and HDL cholesterol [21], and triacylglycerols [22]. Mean bias for control serum provided by the Centers for Disease Control and Prevention (Atlanta, USA) was −1 percent for total and high-density lipoprotein (HDL) cholesterol, and 10 percent for triacylglycerols. The coefficient of variation within runs ranged from 0.9 to 1.7 percent. LDL was calculated by using the Friedewald method [23]. Creatinine was measured with a modified Jaffé method

using a Spectrum kit [Abbott Laboratories, North Chicago, IL, USA) [24]. Concentrations of alanine aminotransferase and aspartate aminotransferase [25], γ-glutamyltransferase [26] and alkaline phosphatase [27] were measured at 37°C using Abbott Spectrum reagents. The mean bias for these enzymes in Monitrol control serum (Baxter Dade, Düdingen, Switzerland) ranged from −1 to 3 percent. The coefficient of variation within runs ranged from 2 to 8 percent. 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 γ-glutamyltransferase. All samples from one subject were analysed in the same run, except for serum samples obtained at the end the second follow-up period (day 183) which were analysed in a separate run.

Statistics

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 analysed by subtracting the values obtained after the preceding run-in period from the treatment values, and the effects of the mixture relative to cafestol alone were analysed by subtracting the values after cafestol from those after the mixture.

Three subjects were switched to placebo halfway through the treatment (see: Design and subjects). 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 amounted 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 γ-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 as end values, as well as analyses without these three subjects. All variables were normally distributed, and none showed significant carry-over or period effects. Differences were therefore tested against zero with one-sided paired t tests [28].

RESULTS

According to the diaries kept by the subjects, more than 99 percent 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 less then 1 consumption per day. The mean change (± SD) in body weight was 0.3 ± 0.8 kg (range −0.4 to 2.3 kg) over the first, and −0.7 ± 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 (± SEM) concentration of total cholesterol by 0.79 ± 0.14 mmol/L ($P<0.001$), LDL – cholesterol by 0.57 ± 0.13 ($P=0.002$), and fasting triacylglycerols by 0.65 ± 0.12 mmol/L ($P<0.001$); it reduced HDL cholesterol by 0.06 ± 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 ± 0.16 mmol/L ($P=0.08$) and LDL cholesterol by 0.23 ± 0.16 mmol/L ($P=0.09$), and triacylglycerols by 0.09 ± 0.10 mmol/L ($P=0.20$) (table 1, figure 2).

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 ± 0.15 mmol/L for total cholesterol ($P=0.15$), 0.15 ± 0.15 mmol/L for LDL cholesterol ($P=0.16$), and 0.08 ± 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 ± 0.19 mmol/L ($P=0.003$), LDL – cholesterol by 0.74 ± 0.13 mmol/L ($P<0.001$), and triacylglycerols by 0.64 ± 0.10 mmol/L ($P<0.001$), relative to baseline. Relative to cafestol alone, the mixture raised total cholesterol by 0.08 ± 0.16 mmol/L ($P=0.31$), LDL cholesterol by 0.06 ± 0.16 mmol/L ($P=0.36$), and triacylglycerols by 0.05 ± 0.12 mmol/L ($P=0.34$) ($n=7$).

TABLE 1. Serum concentrations of lipids, lipoproteins, liver enzymes, and creatinine in 10 healthy male volunteers after consumption of 61 – 64 mg/day of cafestol alone or of a mixture of 60 mg/day of cafestol plus 48 – 54 mg/day of kahweol for 28 days each in a cross – over study [1,2].

	CAFESTOL TREATMENT			MIXTURE TREATMENT			MIXTURE MINUS CAFESTOL	
	Pre – treatment	Post-treatment	Change[3]	Pre-treatment	Post-treatment	Change[3]	Difference [95% CI]	*P*
Total cholesterol (mmol/L)[4]	4.80 ± 0.99	5.59 ± 1.05	0.79 ± 0.44***	4.88 ± 0.89	5.82 ± 1.01	0.94 ± 0.61***	0.23 [−0.05 to 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 to 0.04]	0.20
LDL cholesterol (mmol/L)[5]	2.96 ± 0.78	3.53 ± 0.87	0.57 ± 0.41**	3.05 ± 0.73	3.75 ± 0.77	0.71 ± 0.49**	0.23 [−0.06 to 0.51]	0.09
Triacylglycerols (mmol/L)	0.75 ± 0.21	1.40 ± 0.38	0.65 ± 0.38***	0.77 ± 0.27	1.48 ± 0.61	0.71 ± 0.39***	0.09 [−0.09 to 0.26]	0.20
Alanine aminotransferase (U/L)	23 ± 5	41 ± 10	18 ± 7***	30 ± 9	76 ± 40	46 ± 37**	35 [16 to 54]	0.004
Aspartate aminotransferase (U/L)	22 ± 6	27 ± 5	5 ± 3***	27 ± 9	40 ± 13	12 ± 15*	12 [6 to 18]	0.002
γ – Glutamyltransferase (U/L)	17 ± 6	16 ± 5	−1 ± 2*	19 ± 7	16 ± 6	−3 ± 2***	−0 [−1 to 0]	0.19
Alkaline phosphatase (U/L)	78 ± 21	76 ± 27	−1 ± 11	77 ± 19	72 ± 23	−5 ± 9	−4 [−9 to 1]	0.09
Creatinine (μmol/L)	89 ± 10	86 ± 10	−3 ± 8	90 ± 12	82 ± 9	−8 ± 5***	−4 [−8 to −0]	0.04

[1] Pre – treatment (baseline) values are taken after two weeks on placebo oil, at day 15 for the first and day 106 for the second period. Post – treatment values are taken after diterpene intake, and are averages of days 36, 39, and 43 for the first, and of days 127, 130, and 134 for the second period.

[2] Values are means ± SD

[3] Different from zero: * $P<0.05$, **, $P<0.01$, *** $P<0.001$

[4] To convert mmol/L to mg/dL multiply by 38.67 for cholesterol, and by 88.54 for triacylglycerols.

[5] LDL concentrations were calculated according to Friedewald [23].

Other serum variables

Cafestol alone raised alanine aminotransferase by 18 ± 2 U/L over baseline values ($P<0.001$). Intake of the mixture further raised alanine aminotransferase by 35 ± 11 U/L for the full group of 10 subjects ($P=0.004$) (table 1, figure 2). 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 ± 9 U/L ($P=0.004$). When the three subjects who were switched to placebo were excluded, alanine aminotransferase rose by 18 ± 2 U/L with cafestol alone ($P<0.001$), and by another 20 ± 7 U/L ($P=0.02$) with the mixture (n = 7).

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 ± 2 U/L over baseline values, and two weeks after treatment with the mixture ended, γ-glutamyltransferase had risen by 4 ± 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

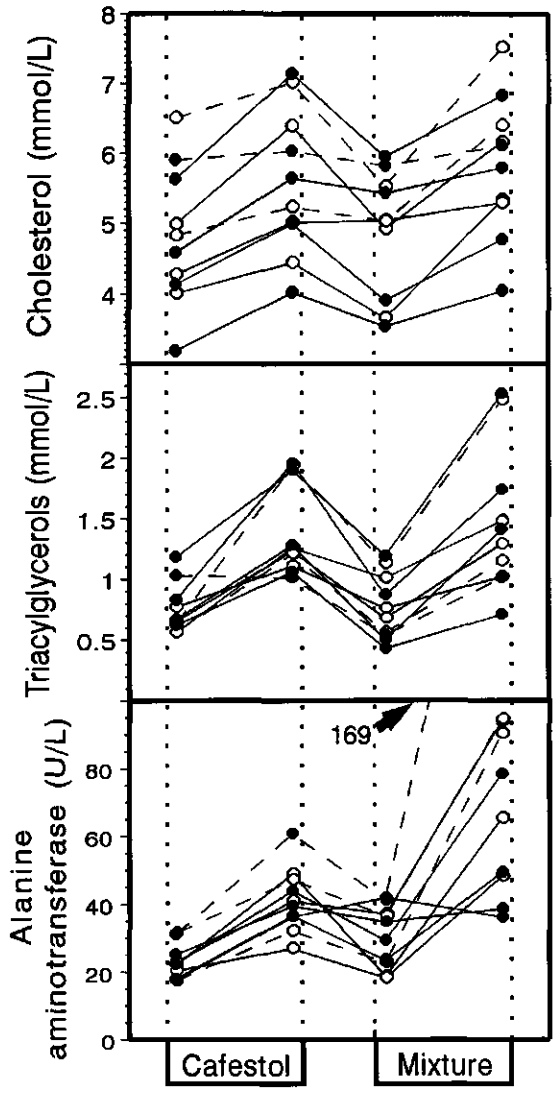

FIGURE 2. Individual baseline values and changes in serum concentrations of cholesterol, triacylglycerols, and alanine aminotransferase after ingestion of either 61 – 64 mg/day of cafestol alone (cafestol treatment), or of a mixture of 60 mg/day of cafestol plus 48 – 54 mg/day of kahweol (mixture treatment) for 28 days each in a cross – over study with ten healthy male volunteers. Subjects indicated by '●' first received cafestol and then the mixture, those indicated by '○' received the opposite sequence. The three subjects who were switched to placebo halfway through the mixture treatment are indicated by broken lines.

imputation from the remaining seven subjects. However, statistical analyses based only on 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 standard deviations of the responses would not [10]. As women tend to respond less to coffee diterpenes than men (unpublished observations), we included only male volunteers in the study so as to further minimize possible variation in responses.

Although we could separate cafestol and kahweol on an analytical scale [11], semi-preparative 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 percent, LDL-cholesterol by 19 percent, and fasting triacylglycerols by 86 percent, whereas consumption of a mixture of cafestol and kahweol further increased total cholesterol by only two percent, LDL-cholesterol by four percent, and triacylglycerols by seven percent. The effect of coffee diterpenes on lipid cholesterol follows a dose-response relation, which appears to be linear up to a dosage of 200 mg of cafestol plus kahweol per day [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 hyperlipidaemic than cafestol.

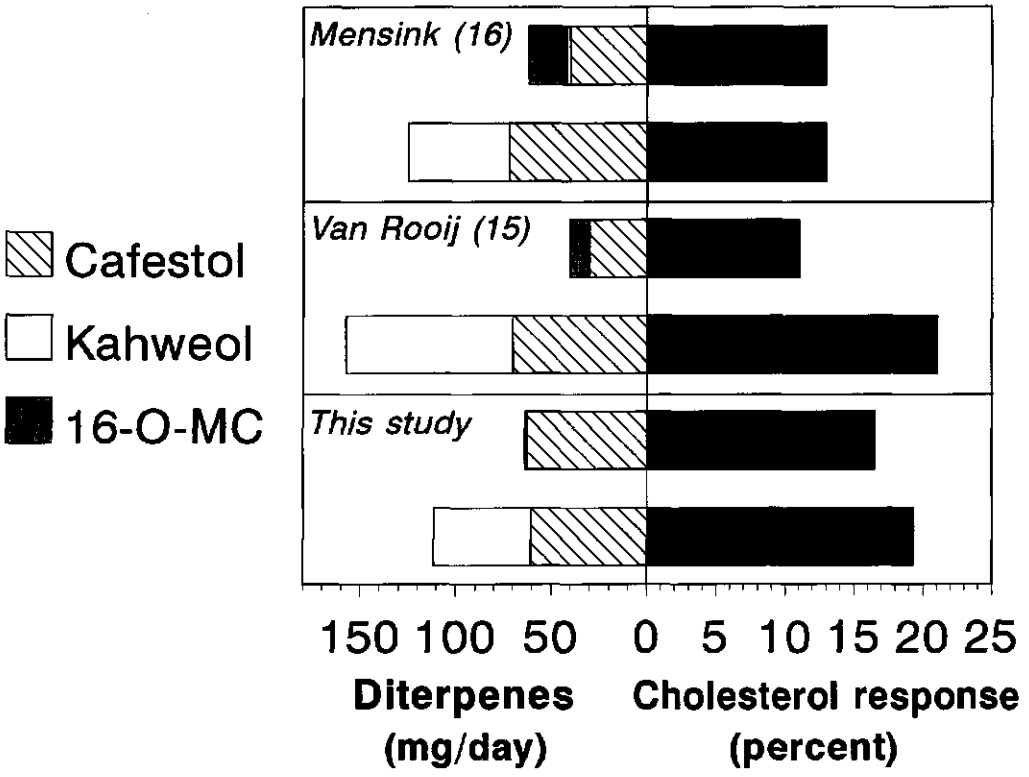

FIGURE 3. Comparison of present results with studies that examined effects of coffee oils containing mixtures of cafestol, kahweol, and 16 – *O* – methylcafestol (16 – O – MC) (left panel) on cholesterol responses (right panel). Mensink et al [16] gave 5 volunteers placebo versus robusta oil (upper bar), and 6 placebo versus arabica oil (lower bar) for 2 x 3 weeks in a cross – over design. Van Rooij et al [15] gave placebo, robusta (upper bar) or arabica oil (lower bar) to three groups of 12 subjects for 6 weeks in a parallel design. We gave 10 volunteers pure cafestol (upper bar) or a mixture of cafestol plus kahweol (lower bar) for 2x4 weeks in a cross – over design.

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 3). Van Rooij et al [15] found that robusta oil increased serum cholesterol by 11 percent, which is consistent with our finding that cafestol is hypercholesterolaemic. 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 percent 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 with our finding that kahweol has only 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 the $C_1 - C_2$ carbon atoms. Possibly, this double bond allows faster biotransformation into compounds that are rapidly excreted, or that have less hyperlipidaemic capacity.

It is not known whether 16 – *O* – methylcafestol – – present in robusta but not in arabica beans [29] – – also affects serum lipoproteins. However, this diterpene accounts for only 3 percent of the diterpenes present in commercial roast and ground coffees [11], and intakes are therefore low.

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 C1 – C2 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 localised 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 γ – glutamyltransferase were reduced upon intake of coffee diterpenes, and showed a rebound increase of about 30 percent after withdrawal of both supplements. A similar pattern was observed in previous studies [10]. The serum concentration of γ – glutamyltransferase is largely determined by the liver, where γ – glutamyltransferase occurs predominantly in

epithelial cells of the biliary duct [13]. The discrepant effects of cafestol and kahweol on γ-glutamyltransferase and alanine aminotransferase suggest that coffee diterpenes (or their metabolites) may have various sites of action in the liver.

Conclusion

Unfiltered coffee brews elevate serum lipoprotein concentrations mainly through their cafestol content, whereas kahweol has little additional effect. Both diterpenes elevate serum liver aminotransferases, indicating that lipid metabolism and liver cell metabolism 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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5

comparison of effect of cafetière and filtered coffee on serum concentrations of liver aminotransferases and lipids

six month randomised controlled trial

ABSTRACT

Objective. *To study the effects of prolonged intake of cafetière coffee, which is rich in the cholesterol-raising diterpenes cafestol and kahweol, on serum aminotransferase and lipid concentrations.*

Design. *Randomised, parallel controlled trial.*

Subjects. *46 healthy men and women, ages 19 to 69.*

Intervention. *Consumption of five to six strong cups (0.9 L) per day of either cafetière (n = 22) or paper-filtered coffee (n = 24) for 24 weeks.*

Main outcome measures. *Mean changes in serum aminotransferase and lipid concentrations.*

Results. *Cafetière coffee raised alanine aminotransferase by up to 80 percent over baseline, relative to filtered coffee. After 24 weeks, the rise was still 45 percent (9 U/L, 95 percent Confidence Interval 3 to 15 U/L, P = 0.007). Alanine aminotransferase exceeded the upper limit of normal in eight, and twice the upper limit in three out of the 22 cafetière coffee consumers. Cafetière coffee raised low-density lipoprotein cholesterol (LDL) concentrations by 9 to 14 percent. After 24 weeks, the rise was 0.26 mmol/L (95 percent Confidence Interval 0.04 to 0.47 mmol/L, P = 0.03), relative to filtered coffee. Triglycerides initially rose by 26 percent on cafetière coffee, but returned close to baseline within half a year. All rises were reversible after treatment was stopped.*

Conclusions. *Daily consumption of five to six cups of strong cafetière coffee affects the integrity of liver cells, as suggested by small increases in serum alanine aminotransferase. The effect does not subside upon prolonged intake. High intakes of coffee brews rich in cafestol and kahweol may thus be responsible for unexplained elevations of this enzyme in apparently healthy individuals. Cafetière coffee also raises low-density lipoprotein cholesterol, and thus coronary heart disease risk (BRITISH MEDICAL JOURNAL 1996; 313: 1362-66).*

INTRODUCTION

Scandinavian boiled coffee raises serum cholesterol concentrations in man [1-4]. The diterpenes cafestol and kahweol are responsible for this effect [5,6]. They do not pass through paper filters, which explains why filtered coffee does not raise blood cholesterol concentrations [7,8]. However, cafestol and kahweol do occur in other unfiltered coffee brews, such as cafetière coffee and Turkish coffee [9].

Cafestol and kahweol seem to affect liver cells; short-term intake of boiled coffee [5] or preparations rich in cafestol and kahweol [5,10-12] raises the serum concentration of alanine aminotransferase (ALT, formerly SGPT). However, life-long consumers of boiled coffee in Norway did not have higher alanine aminotransferase concentrations than matched filtered coffee drinkers [5]. One explanation for this could be that alanine aminotransferase concentrations return to normal with prolonged intake of cafestol and kahweol.

We examined this hypothesis and the effects on serum lipid concentrations in a randomised trial of cafetière versus filtered coffee.

SUBJECTS AND METHODS

Design and subjects

The trial lasted from October 1994 to July 1995. It consisted of a four week run in period, 24 weeks of treatment (intervention), and 12 weeks of follow-up. The study was approved by the local human ethics committee.

Volunteers were recruited through advertisements in newspapers and university buildings. We carefully explained the study protocol to them before they gave written informed consent. Subjects were eligible if they had a Body Mass Index < 30 kg/m^2; did not use any medication known to affect serum liver enzymes or lipids; were not pregnant, lactating, or on a prescribed diet; and drank more than four cups of coffee and less than three drinks containing alcohol per day. Candidates filled out a medical questionnaire, which was reviewed by an independent internist. Candidates with a history of gastro-intestinal, liver, or kidney diseases were excluded, as were those with glucosuria; proteinuria; anaemia; a serum concentration of total cholesterol > 6.5 mmol/L; of fasting triglycerides > 2.3 mmol/L; or concentrations of alanine or aspartate aminotransferase, or γ-glutamyltransferase above the upper limits of normal.

Sixty-four subjects entered a run-in period which served to select those who were able to comply with our requirements. All subjects consumed 0.9 litre of filtered coffee per day. Eleven subjects reported that they could not comply, mainly because they thought the coffee was too strong. We stratified the remaining 53

subjects for sex and alanine aminotransferase concentration (above/below median), and allocated them to either filtered or cafetière coffee by tossing a coin.

In the treatment period, subjects consumed 0.9 litre per day of either filtered or cafetière coffee. They were asked to maintain their usual diet and lifestyle. Intakes of dietary fatty acids and cholesterol were estimated once in the run-in period and three times in the treatment period [13]. All subjects kept daily records of illness and deviations from the protocol. Body weights were measured monthly.

TABLE 1. Characteristics of the participants at the end of the run-in period [a].

Variable	Filtered coffee (n = 24)	Cafetière coffee (n = 22)
Sex (male/female)	12/12	11/11
Age (years) [range]	29 ± 9 [19-52]	30 ± 11 [20-69]
Body Mass Index (kg/m^2)[b]	22 ± 3	23 ± 3
Smoking (yes/no)	10/14	6/16
Women using contraceptives (yes/no)	6/6	5/6
Alcohol use (drinks/day)[c]	1.0 ± 0.6	0.7 ± 1.0
Coffee consumption (cups/day)[c]	5 ± 2	5 ± 1
Cream in coffee (yes/no)	10/14	8/14

[a] Values are numbers, or means ± SD

[b] Body weights were measured without shoes or heavy clothing.

[c] Self-reported consumption prior to the study

Six subjects withdrew during the treatment period; two had problems complying, two became ill, one moved, and one had personal reasons. Another subject was withdrawn after 20 weeks as his aminotransferase concentrations exceeded our predefined limits. He had started daily use of medications of potential hepatotoxicity during the treatment period, and his data were excluded. Inclusion of his values did not materially alter the results (for instance, a rise of alanine aminotransferase after 8 weeks of treatment of 19 instead of 16 U/L). Forty-six subjects completed the study (table 1).

Preparation of coffee

Subjects brewed their coffee at home according to instructions that we gave them before the study. All ground coffee was "Roodmerk" (Douwe Egberts, Utrecht, The Netherlands), a blend of Arabica and Robusta beans widely used in the Netherlands [8].

FILTERED COFFEE. Subjects scooped 78 millilitres (33 grams) of fine grounds into a paper filter (Melitta, Gorinchem, The Netherlands) in a conical holder, which was placed on a 0.5 litre thermos jar. They poured boiling water onto the grounds until the jar was full.

CAFETIÈRE COFFEE. Subjects scooped 92 millilitres (39 grams) of coarse grounds into a cafetière (Kaffee Primo, BMF, Germany, 1 litre), and poured 0.6 litre of boiling water onto the grounds. More coffee was used so as to provide the same amount of caffeine as filtered coffee [14]. Subjects stirred the brew for 10 seconds, allowed it to stand for 2 to 5 minutes, pushed down the screen strainer (plunger) to separate the grounds from the brew, and decanted the brew into a jar.

One jar provided 2 to 3 cups of coffee, and two jars were prepared and consumed each day. Subjects were allowed to dilute the brew with water, if they considered it too strong. Cafetière coffee provided 38 (SD 6) milligrams of cafestol and 33 (SD 5) milligrams of kahweol per day (mean of 22 samples). Filtered coffee provided less than 1 milligram of either diterpene per day (mean of 6 samples).

Blood sampling and assays

Venous blood samples were taken after an overnight fast after 3 and 4 weeks in the run-in period, after 2, 4, 6, 8, 12, 16, 20, 23 and 24 weeks of treatment, and after 4, 8 and 12 weeks in the follow-up period. Sera were obtained by centrifugation, and stored at $-80°C$. Alanine and aspartate aminotransferase [15], alkaline phosphatase [16], and γ-glutamyltransferase [17] were measured at 37°C using Abbott Spectrum reagents. The mean bias for 'Monitrol' control sera (Baxter Dade, Switzerland) ranged from 0 to 2 percent. The coefficient of variation within runs ranged from 2 to 8 percent. Upper limits of normal were 54 U/L for alanine aminotransferase, 40 U/L for aspartate aminotransferase, 92 U/L for

alkaline phosphatase, and 63 U/L and 35 U/L for γ-glutamyltransferase in men and women, respectively. Sera were analysed enzymatically for total [18] and high-density lipoprotein cholesterol [19], and triglycerides [20]. Mean bias for control sera provided by the Centers for Disease Control (Atlanta, USA) was -1 percent for total and high-density lipoprotein cholesterol, and 10 percent for triglycerides. The coefficient of variation within runs ranged from 0.9 to 1.7 percent. Low-density lipoprotein cholesterol was calculated [21].

Alanine aminotransferase and cholesterol were analysed in three separate sessions, each comprising 12 weeks of the trial. All other variables were analysed in a single session. Sera from one subject were analysed within the same run.

Statistics

Baseline values were calculated as the means of the two values obtained after the run-in period. Responses were calculated by subtracting baseline values from values obtained during the treatment period. The means of the values obtained after 23 and 24 weeks of treatment were used as end values. Differences in responses between the groups were compared using Mann Whitney U and unpaired *t* tests. As the results were similar, only the latter are presented.

RESULTS

Diaries kept by the subjects and anonymous questionnaires administered after the trial showed that over 98 percent of the prescribed amount of coffee was consumed. In both groups, mean changes in body mass index during treatment were less than 0.5 kg/m^2, and changes in intake of saturated, mono-unsaturated, or poly-unsaturated fatty acids less than 1 percent of energy.

Liver enzymes

The mean alanine aminotransferase concentration rose on cafetière coffee by up to 80 percent (table 2). After 24 weeks, the rise was 9 (SE 3) U/L or 45 percent over baseline, relative to filtered coffee ($P=0.007$). In eight out of 22

subjects drinking cafetière coffee, the concentration of alanine aminotransferase exceeded the upper limit of normal at least one occasion, as opposed to one out of 24 subject drinking filtered coffee. Alanine aminotransferase exceeded twice the upper limit of normal in three cafetière coffee drinkers. Concentrations of aspartate aminotransferase, alkaline phosphatase, and γ-glutamyltransferase were less affected (figure 1). After discontinuation of cafetière coffee, enzyme concentrations returned to normal in all subjects.

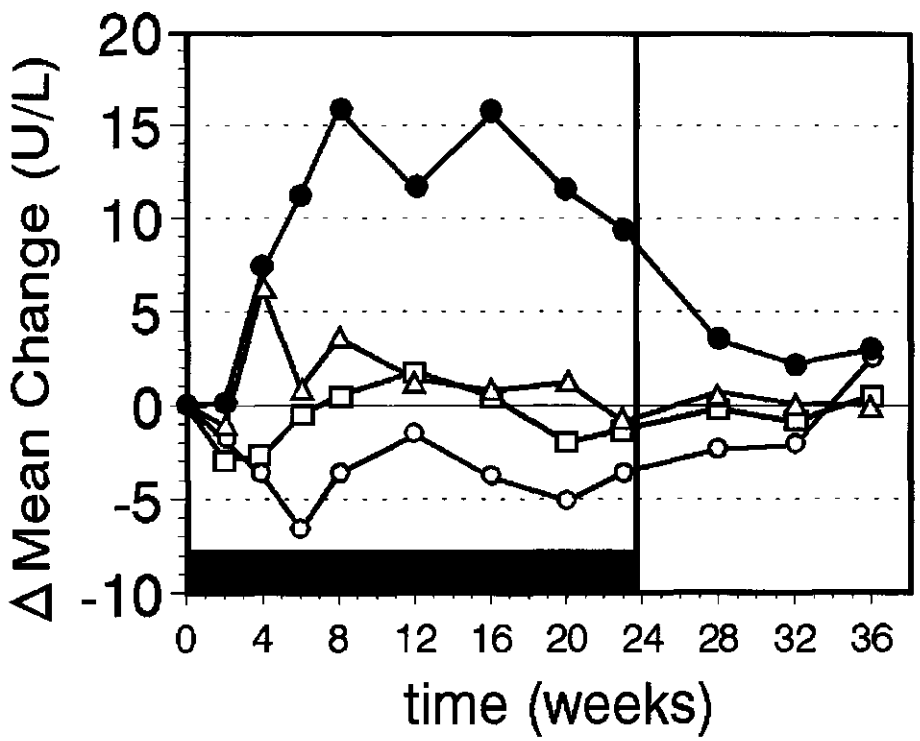

FIGURE 1. Mean changes from baseline values in serum concentrations of alanine (●) and aspartate aminotransferase (△), alkaline phosphatase (○) and γ-glutamyltransferase (□) in 22 subjects drinking 0.9 litre per day of cafetière coffee for 24 weeks. For each time point, the mean changes from baseline occurring in 24 subjects drinking filtered coffee were subtracted from the mean changes in cafetière coffee consumers, to correct for random drifts in time. The treatment period is indicated by a horizontal black bar. No treatment was given in the follow-up period.

TABLE 2. Mean serum levels of alanine aminotransferase and lipids before, during, and after consumption of 0.9 litre per day of filtered (n = 24) or cafetière coffee (n = 22) for half a year. Baseline values were obtained after subjects had consumed filtered coffee for 3-4 weeks. No treatment was given in the follow-up period.

		TREATMENT PERIOD							FOLLOW-UP			
	Baseline	*wk 4*	*wk 8*	*wk 12*	*wk 16*	*wk 20*	*wk 24*	*Treatment effect (95% CI)*[a]	*wk 28*	*wk 32*	*wk 36*	*wk 64*[b]
Serum variable												
Alanine aminotransferase (U/L)												
Filtered	20	21	22	21	21[c]	20	19		18[c]	18[c]	18[c]	19
Cafetière	19	27	36[c]	31	35	30	27	9 (3 to 15)	20	19	19	15
Total cholesterol (mmol/L)[d]												
Filtered	4.99	5.04	5.13	5.08	4.79[c]	4.94	4.94		4.97[c]	5.06[c]	4.94[c]	5.25
Cafetière	4.91	5.35	5.56[c]	5.52	5.24	5.26	5.16	0.31 (0.01 to 0.61)	4.62	4.68	4.63	4.88
Low density lipoprotein cholesterol (mmol/L)[d,e]												
Filtered	2.99	3.03	3.09	3.06	2.76[c]	2.93	2.92		2.98[c]	2.97[c]	2.89[c]	3.20
Cafetière	2.99	3.29	3.51[c]	3.42	3.24	3.28	3.18	0.26 (0.04 to 0.47)	2.78	2.76	2.78	2.92
Triglycerides (mmol/L)[f]												
Filtered	1.05	1.11	1.14	1.05	1.05[c]	1.07	1.18		1.05[c]	1.16[c]	1.26[c]	1.19
Cafetière	1.07	1.41	1.32[c]	1.34	1.31	1.16	1.26	0.07 (-0.23 to 0.37)	0.96	1.03	1.01	1.03

[a] After 23-24 weeks; calculated by subtracting the mean change from baseline on filtered coffee from that on cafetière coffee
[b] Based on 20 subjects who had received cafetière coffee, and 15 who had received filtered coffee
[c] Value of one subject missing
[d] Calculated according to Friedewald [21].
[e] To convert to mg/dL multiply by 38.67
[f] To convert to mg/dL multiply by 88.54

Lipids

Cafetière coffee raised cholesterol concentrations, mostly because of an increase in low-density lipoprotein cholesterol (figure 2, table 2). After 24 weeks, low-density lipoprotein cholesterol concentrations were raised by 0.26 (SE 0.11) mmol/L or 9 percent over baseline, relative to filtered coffee (P=0.03). High-density lipoprotein cholesterol was not affected. Triglycerides were raised by 26 percent within two weeks, and by 7 percent after 24 weeks of treatment. After discontinuation of cafetière coffee, concentrations of total and low-density lipoprotein cholesterol, and of triglycerides fell below baseline (figure 2).

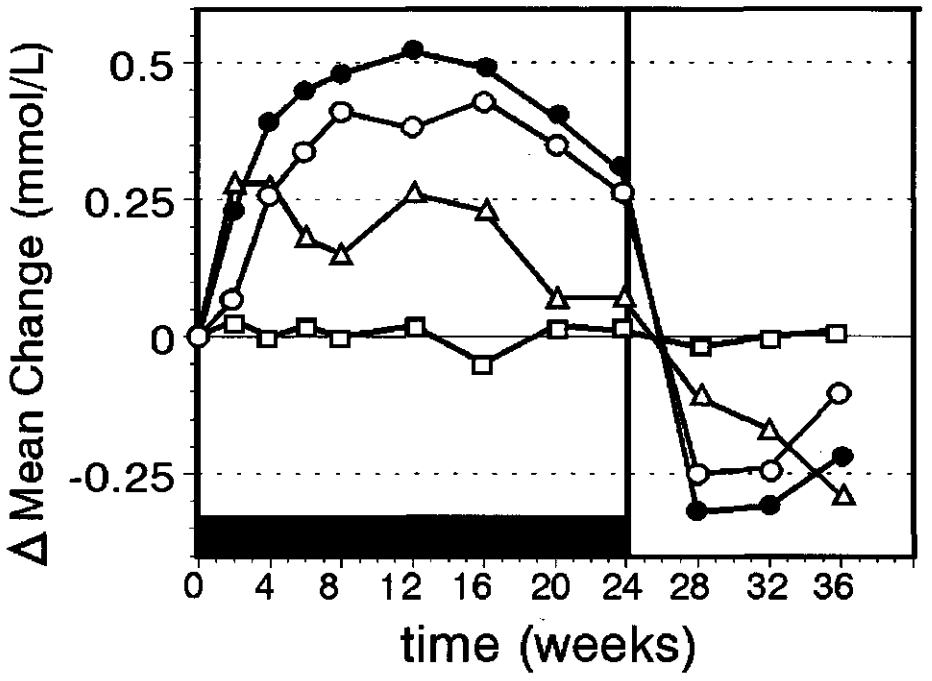

FIGURE 2. Mean changes from baseline in serum concentrations of total cholesterol (●), triglycerides (△), low-density lipoprotein cholesterol (LDL,○) and high-density lipoprotein cholesterol (HDL,□) in 22 subjects drinking 0.9 litre of cafetière coffee per day for 24 weeks. For each time point, the mean changes from baseline occurring in 24 subjects drinking filtered coffee were subtracted from the changes in the cafetière coffee consumers, to correct for random drifts in time. The treatment period is indicated by a horizontal black bar. No treatment was given in the follow-up period. Low-density lipoprotein cholesterol was calculated according to Friedewald [21].

DISCUSSION

We found that daily intake of five to six cups of strong cafetière coffee raises alanine aminotransferase concentrations for at least half a year, and raises low-density lipoprotein cholesterol similarly to Scandinavian boiled coffee. The present findings should also apply to Turkish coffee, which contains similar amounts of cafestol and kahweol per cup as cafetière and boiled coffee. For Italian espresso coffee, about twenty cups per day are required for these effects, due to small cup sizes. Instant and percolator coffee will have negligible effects on serum aminotransferase and lipid concentrations, due to their very low concentrations of cafestol and kahweol [9].

Liver enzymes

We expected that the rise in alanine aminotransferase would be transient with prolonged intake of cafetière coffee, as chronic consumers of boiled coffee did not have elevated alanine aminotransferase concentrations [5]. However, the present study indicates that liver cells only partly adapt to cafestol and kahweol within the first half year of consumption.

Alanine aminotransferase concentrations exceeded the upper limit of normal in one out of three, and twice the upper limit in one out of seven subjects on cafetière coffee. Thus, a high intake of strong, unfiltered coffee might explain some cases of elevated alanine aminotransferase concentrations in apparently healthy individuals. It might be prudent for patients with raised alanine aminotransferase not to drink more than a few cups of cafetière, Turkish, or boiled coffee per day on a regular basis.

However, should we really expect unfiltered coffee to affect the risk of liver disease in healthy individuals? Cafetière coffee only marginally raised aspartate aminotransferase concentrations, which excludes extensive damage to liver cells. γ-Glutamyltransferase and alkaline phosphatase concentrations were reduced rather than raised (figure 1), and reduced concentrations of γ-glutamyltransferase were also observed in chronic consumers of boiled coffee [22,23]. In Scandinavian countries, which used to have high intakes of boiled coffee, death rates from liver cirrhosis are low, and seem to be unaffected by the change from drinking boiled

to drinking filtered coffee over the past decades [24]. Therefore, clinically relevant damage to liver cells with regular use of unfiltered coffee appears unlikely, although we cannot yet fully exclude subclinical injury to hepatocytes.

Lipids

Cafetière coffee raised total cholesterol by 6 to 10 percent, and low-density lipoprotein cholesterol by 9 to 14 percent. This is similar to the effects observed in experiments with Scandinavian boiled coffee [2-4,7,8].

The rises in total and low-density lipoprotein cholesterol persisted with prolonged consumption of cafetière coffee. Our experiment thus shows that cholesterol metabolism remains disturbed with prolonged intake of cafestol and kahweol, as was previously suggested by observational studies in boiled-coffee drinkers [5,25-28]. Therefore, high intakes of cafetière coffee will be associated with an increased risk of coronary heart disease, similar to boiled coffee [29]. An elevation of cholesterol of 6 to 10 percent is estimated to increase coronary risk by 12 to 20 percent [30]; larger increases may be expected in young individuals [31].

High triglyceride concentrations are also associated with increased coronary risk [32]. Triglycerides initially rose on cafetière coffee by 26 percent, but most of the rise disappeared with prolonged intake (figure 2). It is unlikely that this was due to seasonal influences, as we expressed all changes relative to those in the concurrent control group. Experiments with boiled coffee [2,3,7,8] have shown larger responses in triglycerides than observational studies comparing consumers of boiled and filtered coffee [4,27]. Our results may explain this; triglyceride concentrations partly normalize with long-term intake of cafestol and kahweol.

We cannot explain the fall in lipid concentrations below baseline values after cessation of cafetière coffee. It is not likely to be due to dietary changes; for instance, the observed reduction of low-density lipoprotein cholesterol of 0.25 mmol/L would have required a shift in dietary intake from butter to high-linoleic-acid diet margarine of 32 grams per day [33,34]. These findings again emphasize the prolonged and extensive effects of cafestol and kahweol on lipid metabolism in humans.

Conclusions

Daily consumption of large amounts of cafetière coffee raises alanine aminotransferase concentrations for at least half a year. Chronic intake of cafetière coffee also raises low-density lipoprotein cholesterol. The effects on aminotransferases may be innocuous, but those on cholesterol will increase coronary risk, and could be a reason to advise patients to switch to filtered coffee.

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6

chronic consumers of boiled coffee have elevated serum levels of lipoprotein(a)

ABSTRACT

Objectives. *Lipoprotein(a) consists of an LDL-particle attached to apolipoprotein(a), which is made by the liver. Diterpenes present in boiled coffee raise serum levels of LDL cholesterol and of the liver enzyme alanine aminotransferase in man. We now investigated the association between intake of boiled coffee and serum levels of lipoprotein(a).*

Design, Setting, and Subjects. *Healthy Norwegians 40-42 y of age, who habitually consumed five or more cups of boiled coffee per day (n = 150) were compared with matched filter coffee consumers (n = 159) in a cross-sectional study, as part of the Norwegian National Health Screening in 1992.*

Results. *The median lipoprotein(a) level was 13.0 mg/dL (10th and 90th Percentile: 2.5 and 75.0 mg/dL, respectively) on boiled and 7.9 mg/dL (10th and 90th Percentile: 1.9 and 62.5 mg/dL, respectively) on filter coffee (P = 0.048). Means ± SE were 25.8 ± 2.4 mg/dL and 19.6 ± 2.0 mg/dL, respectively (P = 0.047). Although not statistically significant, subjects consuming nine or more cups of coffee per day had higher lipoprotein(a) levels than those drinking five to eight cups per day in both coffee groups.*

Conclusion. *Chronic consumers of unfiltered, boiled coffee have higher serum lipoprotein(a) levels than filter coffee drinkers (JOURNAL OF INTERNAL MEDICINE 1996; 240: 367-71).*

INTRODUCTION

Lipoprotein(a) [Lp(a)] consists of a large glycoprotein, apolipoprotein(a), attached to an LDL molecule [1]. A high serum Lp(a) level is a risk factor for developing coronary atherosclerosis and other vascular diseases [1-5]. Lp(a) levels are largely under genetic control [6], and are unaffected by many factors that modulate LDL-cholesterol levels [1,7], including diet [8]. An exception is formed by dietary *trans* fatty acids, which raise serum Lp(a) levels [9,10].

Consumption of unfiltered, boiled coffee is associated with raised serum cholesterol levels in humans in both intervention trials [11-13] and epidemiological studies [14-17]. Recently, it was shown that the diterpenes cafestol and kahweol are responsible for the hyperlipidaemic effects of boiled coffee [18,19]. Scandinavian boiled coffee provides 3 to 4 mg of each diterpene per cup, whereas paper-filtered coffee contains less than 0.1 mg of each diterpene per cup [20].

The liver appears to be the target organ of coffee diterpenes, as cafestol and

kahweol elevate the serum activity of alanine aminotransferase [19,21] and reduce that of γ-glutamyltransferase [19,22]. The liver is also the main source of apolipoprotein(a) [23], and the serum level of Lp(a) is largely determined by the rate of Lp(a) production [24]. Whether ingestion of coffee diterpenes with boiled coffee results in altered serum levels of Lp(a) is as yet unknown.

We now compared serum Lp(a) levels in healthy Norwegians consuming unfiltered boiled coffee with those in matched filter coffee drinkers.

TABLE 1. Characteristics of 309 healthy Norwegians aged 40-42 y chronically drinking five or more cups of boiled or filter coffee per day[a]

	Filter coffee *n = 159*	Boiled coffee *n = 150*
Number of men/women	88/71	79/71
Age (years)	41 ± 1	41 ± 1
Body Mass Index (kg/m^2)	25 ± 3	25 ± 4
Women taking contraceptive steroids	2	2
Coffee consumption		
5-8 cups per day	133	117
9 or more cups per day	26	33
Smokers	89	87
Alcohol users	146	135
Medication users[b]	20	21
Total cholesterol (mmol/L)	5.67 ± 1.05	5.98 ± 1.08[c]
Triglycerides (mmol/L)	1.92 ± 1.24	2.14 ± 1.55
Alanine aminotransferase (U/L)	19 ± 10	20 ± 10
Aspartate aminotransferase (U/L)	22 ± 7	22 ± 7
γ-Glutamyltransferase (U/L)	26 ± 24	20 ± 17[c]

[a] Values are numbers, or means ± SD

[b] Any medications used in the month previous to the screening. None of the subjects used medicines on a daily basis.

[c] Significantly different from filter coffee group: $P < 0.05$

SUBJECTS AND METHODS

Subjects

Subjects were recruited as part of the Norwegian National Health Screening in 1992, a population-based study in southern Norway [17]. Persons aged 40-42 years attending the screening were asked to fill out a questionnaire concerning their coffee usage. Those who reported drinking 5 or more cups of either boiled or filter coffee per day received an oral explanation about the purpose of our study. The questionnaire did not provide information on duration and history of coffee intake. Subjects were considered eligible if they were healthy, did not take any medication known to affect liver enzymes or serum lipids, and did not consume more than 3 alcohol-containing beverages per day. Those who were willing to participate gave their written informed consent. Prior approval for the study was obtained from the appropriate human ethics committee.

Blood sampling and assays

Non-fasting blood was obtained by venepuncture, allowed to clot, and centrifuged. Sera were randomly coded, shipped to Wageningen, and stored at -80°C. Lp(a) was assayed blindly within 12 months with an enzyme-linked immunosorbent assay (Immunozym Lp(a), Immuno GMBH, Heidelberg, Germany). The lower limit of detection of Lp(a) was 1 mg/dl, which according to the manufacturer is higher than the maximum cross-reactivity of the Lp(a)-antibodies with plasminogen and apolipoprotein B. For each subject, Lp(a) was analysed in duplicate in separate runs. The intra- and inter-assay coefficients of variation for a control pool (target 14-21 mg/dL) were 14.8 and 9.6 %, respectively, over a period of 3 months. Serum lipids and serum activities of liver enzymes were assayed as described [19].

Statistical analyses

Unpaired, two-sided *t* tests were used to compare the means of serum variables between the two groups. As the distributions of Lp(a) levels were positively skewed, we also compared the two groups with Mann-Whitney U tests.

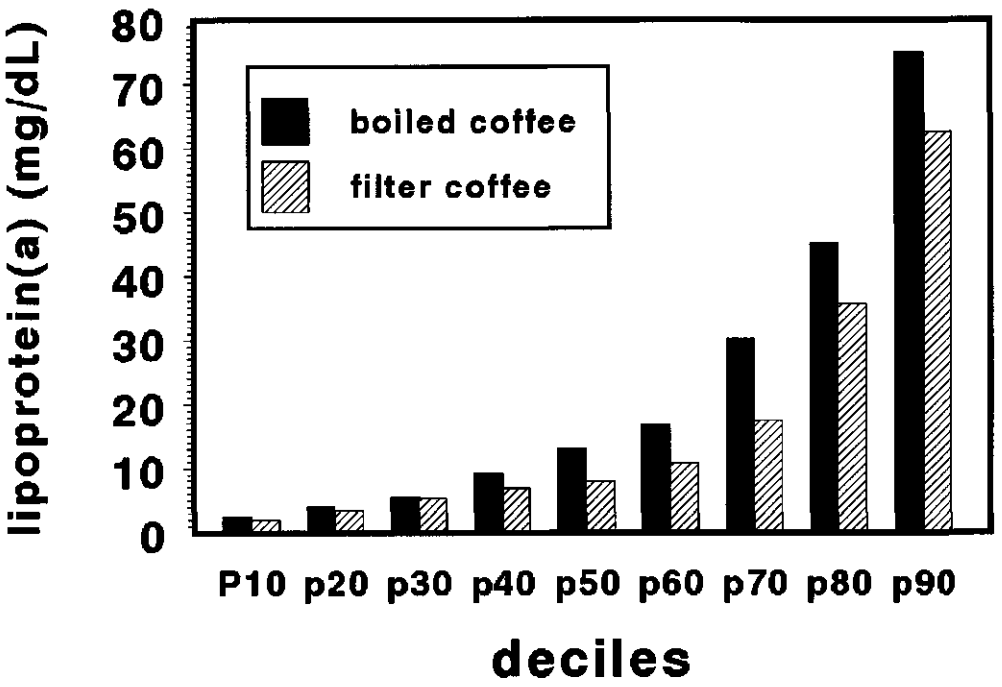

FIGURE 1. Serum lipoprotein(a) levels among 309 healthy Norwegians aged 40-42 y, chronically consuming five or more cups of boiled (n = 150) or filter coffee (n = 159) per day. Values represent the serum Lp(a) level of the 15th, 30th, 45th etc. consumer of boiled coffee, and the 16th, 32nd, 48th, etc. consumer of filter coffee when subjects were ranked for the serum level of Lp(a) within coffee usage groups.

RESULTS

Consumers of boiled (n = 150) and filter coffee (n = 159) were well matched with regard to sex, age, Body Mass Index, use of medicines, smoking habits and

alcohol consumption (table 1). The use of boiled coffee was associated with higher serum levels of cholesterol and lower serum activities of γ-glutamyltransferase, in agreement with earlier findings [14-17,25].

The median level of Lp(a) was 13.0 mg/dL (range 0 to 130 mg/dL) among consumers of boiled coffee and 7.9 mg/dL (range 0 to 144 mg/dL) among consumers of filter coffee ($P=0.048$) (figure 1); means ± SE were 25.8 ± 2.4 mg/dL and 19.6 ± 2.0 mg/dL, respectively ($P=0.047$).

The median Lp(a) levels were for men 14.1 mg/dL for boiled and 7.8 mg/dL for filter coffee, and for women 10.7 mg/dL for boiled and 8.3 mg/dL for filter coffee. The median Lp(a) level was 13.6 mg/dL in the subjects drinking more than nine cups of boiled coffee per day, and 11.7 mg/dL in those drinking less than nine cups. For filter coffee, the corresponding values were 8.0 mg/dL, and 6.9 mg/dL, respectively (all differences $P>0.05$).

DISCUSSION

We found that the median level of serum Lp(a) in Norwegians habitually consuming unfiltered, boiled coffee was significantly higher than in their peers drinking filter coffee. Our data also suggest that the elevation of Lp(a) may depend on the amount of coffee consumed, and that the effect of boiled coffee is stronger in men than in women. These results did not reach statistical significance, however, which may be attributable to reduced power due to limited sample sizes.

The insensitivity of Lp(a) levels to environmental influences [7,8] renders it unlikely that our finding was biased. In addition, the two groups were well matched with respect to age, sex, Body Mass Index, smoking habits, and alcohol use. The use of drugs or contraceptive pills during the previous month in the two groups was infrequent and similarly distributed (table 1). Dietary factors that influence Lp(a) levels are limited to *trans* fatty acids [9,10] and possibly fish oils [8]. It is unlikely, however, that consumers of boiled and filter coffee differed enough in their intake of fatty acids to explain the effect seen here.

The Lp(a) particle contains apolipoprotein(a) [apo(a)], which is remarkably

polymorphic [26]. Serum Lp(a) levels correlate inversely with the molecular weight of the apo(a) isoform and the size of the corresponding gene [27]. We had no information on apo(a) phenotype distribution of the two groups. Although all subjects were living in Southern Norway, more people consuming boiled coffee may have descended from ancestors that originated from Northern Norway -- where consumption of boiled coffee is more common [17]. Therefore, confounding by genetic differences can not be fully excluded.

The higher level of Lp(a) among boiled-coffee consumers was an unexpected finding to us. In four experiments with a large number of Dutch volunteers, coffee oil rich in diterpenes significantly lowered serum Lp(a) (Chapter 7). In the present study, serum levels of cholesterol were higher in the boiled-coffee group, and γ-glutamyltransferase activities were lower, which is consistent with both epidemiological [14-17,25] and experimental studies [19,22]. These variables, as well as the Lp(a) concentration of each subject were determined blindly in the same set of serum aliquots. In addition, all sera of subjects consuming boiled or filter coffee had been stored under the same conditions, and analysed for Lp(a) in random order, except for one analysis run which after breaking of the code turned out to have comprised sera of 36 boiled-coffee drinkers and no filter coffee drinkers, and one run that had contained sera of 42 filter coffee drinkers and only 9 boiled-coffee drinkers. However, the median level of Lp(a) in the first run was 11.5 mg/dL (n = 36) against 13.0 mg/dL for the entire boiled-coffee group (n = 150), and in the second run 8.6 mg/dL (n = 42) against 7.9 mg/dL for the entire filter coffee group (n = 159). Therefore, if any systematic bias was introduced at this stage, it should have weakened the association between coffee type and Lp(a) levels instead of having strengthened it.

Therefore, there seems to be a discrepancy between effects on Lp(a) levels with short-term and chronic intake of coffee diterpenes. In our short-term trials, we observed that coffee diterpenes increased serum activities of alanine aminotransferase [19,22]. This suggests that the liver is the target organ for coffee diterpenes. Disturbed hepatocyte integrity may reduce circulating Lp(a) levels, as seen in patients with biliary cirrhosis or other liver disease [28,29]. In the present study, however, the boiled-coffee drinkers had no elevated serum activities of alanine aminotransferase ([19], see also table 1). It is therefore possible that the

liver adapts to the effects of cafestol and kahweol with chronic consumption of boiled coffee. Therefore, intervention trials of longer duration are needed to reveal the time course of the effect of coffee diterpenes on serum Lp(a).

Tverdal et al [30] found an effect of boiled-coffee consumption on mortality from coronary heart disease, over and above its effect on serum cholesterol. Higher serum levels of Lp(a) on boiled coffee as observed in our study might partly account for this additional risk.

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7

diterpenes from coffee beans reduce serum levels of lipoprotein(a) in humans

results from four randomised controlled trials

ABSTRACT

Objective. *Unfiltered coffee raises serum LDL cholesterol in humans, owing to the presence of the diterpenes cafestol and kahweol. Norwegians with a chronic high intake of unfiltered coffee also had elevated serum levels of lipoprotein(a), an LDL-like particle which is insensitive towards dietary interventions. We now experimentally studied the influence of coffee diterpenes on lipoprotein(a) levels.*

Design. *Four randomised controlled trials.*

Subjects. *Healthy, normolipidaemic volunteers.*

Interventions. *Coffee, coffee oil, and pure diterpenes for 4 to 24 weeks.*

Main outcome measures. *The circulating level of lipoprotein(a).*

Results. *In 22 subjects drinking five to six strong cups of cafetière coffee per day, the median fall in lipoprotein(a) was 1.5 mg/dL after two months ($P=0.03$), and 0.5 mg/dL after half a year ($P>0.05$), relative to 24 filter coffee drinkers. Coffee oil doses equivalent to 10-20 cups of unfiltered coffee reduced lipoprotein(a) levels by up to 5.5 mg/dL ($P<0.05$) in two separate trials ($n=$ 12-16 per group). A purified mixture of cafestol and kahweol, as well as cafestol alone, were also effective in reducing Lp(a) levels ($n=10$). Averaged over the four trials, each 10 mg/day of cafestol (plus kahweol) -- the amount present in two to three cups of cafetière coffee -- decreased Lp(a) levels by 0.5 mg/dL or 4% from baseline values after four weeks ($n=63$).*

Conclusions. *Coffee diterpenes are among the few dietary exceptions shown to influence serum lipoprotein(a) levels. However, the Lp(a)-reducing potency of coffee diterpenes may subside in the long run, and their adverse side effects preclude their use as lipoprotein(a)-reducing agents (EUROPEAN JOURNAL OF CLINICAL NUTRITION - IN PRESS).*

INTRODUCTION

Lipoprotein(a) [Lp(a)] is a liver-derived lipoprotein particle that is only distinguished from low-density-lipoprotein (LDL) by the covalent attachment of a large glycoprotein, apolipoprotein(a) [1]. An extensive body of laboratory evidence indicates a critical role of Lp(a) in the development of atherothrombotic diseases [2], which is confirmed by most [3-10], though not all [11-14], prospective cohort studies. The circulating level of Lp(a) is largely under genetic control [15], and is unaffected by many interventions known to affect LDL metabolism, including most dietary interventions [16].

The diterpenes cafestol and kahweol are lipids that are unique to coffee beans [17]. They are responsible for the cholesterol-raising effect of Scandinavian boiled coffee [18,19]. Cafestol and kahweol strongly affect lipid metabolism with short-term intake [20-25] as well as in life-long consumers of unfiltered coffee [18,26-29]. We recently found that consumption of boiled coffee was also associated with Lp(a) levels; the median Lp(a) level in Norwegians who were life-long consumers of boiled coffee was 65% higher than in matched filter coffee drinkers [30].

We now report the effects of preparations rich in cafestol and kahweol on Lp(a) levels in healthy, normolipidaemic volunteers in four independent experiments. The effects on serum lipids and liver enzymes have been reported elsewhere [18,25,31].

TABLE 1. Characteristics of the participants of the four randomised, controlled trials. Values were obtained at the end of the run-in periods, when subjects had received five cups of filtered coffee free from diterpenes for four weeks (trial A), or 2-3 g of placebo oil for one or two weeks (trials B-D)[a].

	Trial A	Trial B	Trial C	Trial D
Number of men/women	23/23	15/17	21/25	10/0
Women using contraceptive steroid (n)	11	10	12	--
Smokers (n)	16	5	3	2
Age (years)	29 ± 10	22 ± 2	22 ± 2	24 ± 4
Body Mass Index (kg/m^2)[b]	22 ± 3	22 ± 2	22 ± 2	21 ± 2
Serum cholesterol (mmol/L)	4.9 ± 0.7	4.5 ± 0.5	4.5 ± 0.7	4.8 ± 0.9
Serum LDL cholesterol (mmol/L)	3.0 ± 0.8	2.5 ± 0.5	2.7 ± 0.6	3.0 ± 0.7
Serum HDL cholesterol (mmol/L)	1.5 ± 0.3	1.5 ± 0.3	1.4 ± 0.3	1.5 ± 0.4
Serum triglycerides (mmol/L)	1.1 ± 0.4	1.0 ± 0.3	0.9 ± 0.3	0.8 ± 0.2

[a] Values are numbers, or means ± SD

[b] Body weights were measured without shoes or heavy clothing.

METHODS

Subjects

Participants were recruited through advertisements in newspapers and university buildings. Most subjects were young, nonobese, and normolipidaemic (table 1), and all were apparently healthy as indicated by a medical questionnaire and by the absence of anaemia, glucosuria, or proteinuria. None took medications known to affect serum lipid levels. The experimental protocols, which were approved by the local human ethics committee, were carefully explained to the volunteers and their written informed consent was obtained.

Subjects were asked to maintain their usual diet and life-style, and not to consume more than twenty drinks containing alcohol per week. They were allowed to use paper-filtered or instant coffee, as these are free from diterpenes [32]. All subjects kept daily records of illness, medication use, and deviations from the protocol.

Treatments and designs

Between 1991 and 1995, we carried out four randomised trials. We gave filtered versus unfiltered coffee in trial A, placebo versus coffee oil in trials B and C, and cafestol versus a mixture of cafestol and kahweol in trial D. The experimental designs are given in figure 1.

TRIAL A - UNFILTERED VERSUS FILTERED COFFEE. This was a randomised, parallel controlled study. After a run-in period of four weeks on filtered coffee, subjects consumed 0.9 litre of either filtered or cafetière (also called 'French press') coffee per day for 24 weeks. All ground coffee was Roodmerk (Douwe Egberts, Utrecht, The Netherlands), a blend of Arabica and Robusta beans widely used in the Netherlands [22]. Cafetière coffee provided 38 mg of cafestol and 33 mg of kahweol and filtered coffee less than 1 mg of either diterpene per day. Six subjects withdrew during the treatment period for personal reasons, and one was withdrawn as he started taking drugs of potential hepatotoxicity daily during the trial.

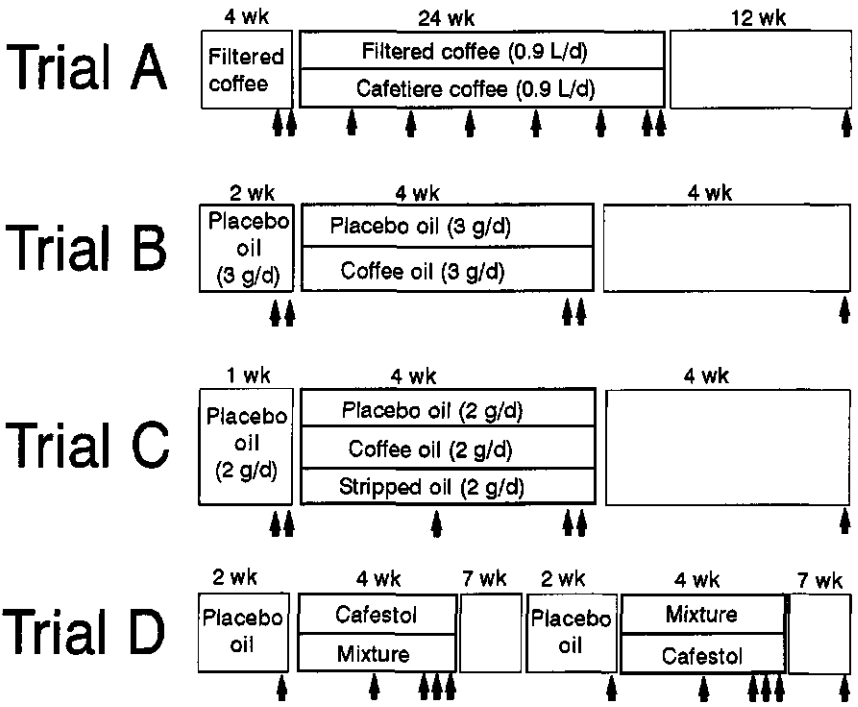

FIGURE 1. Experimental designs of four randomised studies with healthy normolipidaemic volunteers. Arrows indicate times of blood sampling. Empty cells indicate wash-out and follow-up periods, during which no treatment was given.

TRIALS B AND C - COFFEE OIL VERSUS PLACEBO OIL. These were double-blind, randomised parallel controlled studies. All participants first received for 1 to 2 weeks placebo oil which was a 3:2 (w/w) mixture of sunflower oil and palm oil. After randomisation, subjects continued on either placebo oil or coffee oil for four weeks. Daily amounts of diterpenes provided by the coffee oil were 85 mg of cafestol and 103 mg of kahweol in trial B, and 57 mg of cafestol and 69 mg of kahweol in trial C. In trial C, a third group of subjects received coffee oil that was

stripped of cafestol and kahweol.

There were no dropouts in trial B. Two subjects withdrew from trial C for reasons unrelated to treatment.

TRIAL D - CAFESTOL PLUS KAHWEOL VERSUS CAFESTOL ALONE. This was a double-blind, randomised cross-over study. Participants received placebo oil during the first two weeks. During the next four weeks, half of the subjects received 64 mg of cafestol and 1 mg of kahweol dissolved in placebo oil. The other five subjects received 60 mg of cafestol and 54 mg of kahweol per day, again dissolved in placebo oil. After a wash-out period of 7 weeks, subjects again received placebo oil for two weeks, and supplements were switched during the next four weeks; analysed dosages of cafestol and kahweol were now 61 and 0 mg for pure cafestol, and 60 and 48 mg for the mixture, respectively. Purities of the diterpene preparations were higher than 92%; impurities consisted of free cafestol and kahweol, cafestol and kahweol dipalmitate, and palmitic acid.

Three subjects were switched to placebo after two weeks of treatment, because they exceeded our predefined safety limits for rises in alanine aminotransferase. For these subjects, values obtained after two weeks replaced their final values.

Serum analyses

Venous blood samples were taken after an overnight fast. Serum samples were obtained by centrifugation, stored at -80°C, and analysed blindly within 12 months after completion of the studies.

For trials B and C, Lp(a) was measured with a solid phase two-site immunoradiometric assay (RIA) from Pharmacia Diagnostics AB (Uppsala, Sweden). The intra- and inter-assay coefficients of variation as given by the manufacturer were 3.3 and 10.6 %, respectively. The range of measurable serum Lp(a) concentrations was 1.7 to 84.0 mg/dL. Sera with concentrations higher than 84.0 mg/dL were diluted and remeasured. Six out of 78 subjects had Lp(a) levels less than 1.7 mg/dL. These were designated undetectable. The results were recalculated from arbitrary units to milligrams per decilitre on the basis of the results obtained with the kit calibrators, and the value of a serum standard from

Immuno A.G. (Vienna, Austria).

For trials A and D, Lp(a) was measured with an enzyme-linked immunosorbent assay (TintElize Lp(a), Campro Scientific, Veenendaal, the Netherlands). The lower limit of detection of Lp(a) was 1 mg/dL. None of the subjects had levels below the detection limit. The intra- and inter-assay coefficients of variation for a control pool of 44 mg/dL as measured in our laboratory were 5.0 % and 3.5 %, respectively.

For all trials, sera were analysed in duplicate in two separate runs, but with one full series of samples obtained from one subject analysed within the same run. Lipids, lipoproteins, and liver enzymes in serum [18], and cafestol and kahweol in coffee preparations [32], were assayed as described.

Statistical analyses

We calculated the Lp(a) response of each subject by subtracting values obtained after the run-in period (baseline values) from those obtained during the treatment period (treatment values). As some of the responses showed non-Gaussian distributions, we used Mann Whitney U tests to compare control and treatment groups. In trial D, responses were analysed using Wilcoxon Rank Sum tests.

RESULTS

Diaries kept by the subjects showed that more than 98% of the diterpene preparations had been taken in each of the trials.

TRIAL A - UNFILTERED VERSUS FILTERED COFFEE. Daily consumption of five cups of cafetière coffee produced a fall in Lp(a) levels, which reached a maximum of 1.5 mg/dL after eight weeks ($P=0.03$), relative to filtered coffee (figure 2). The reduction stabilised around 0.5 mg/dL ($P>0.05$) between 12 and 24 weeks of consumption.

TRIALS B AND C - COFFEE OIL VERSUS PLACEBO OIL. In trial B, intake of 3 g of coffee oil per day for four weeks caused a median fall in Lp(a) levels of 5.3 mg/dL,

relative to concurrent controls receiving placebo oil ($P<0.001$). In trial C, intake of 2 g/d of coffee oil reduced Lp(a) levels by 3.1 mg/dL ($P=0.02$). Coffee oil that was stripped of cafestol and kahweol did not affect Lp(a) levels (table 2).

TRIAL D - CAFESTOL PLUS KAHWEOL VERSUS CAFESTOL ALONE. The mixture of cafestol and kahweol as well as cafestol alone both reduced Lp(a) levels by 3 to 4 mg/dL relative to baseline levels (table 2). Relative to cafestol alone, the mixture produced a median fall in Lp(a) levels of 0.8 mg/dL, and a mean fall of 0.5 (SEM 0.6) mg/dL (both $P>0.05$).

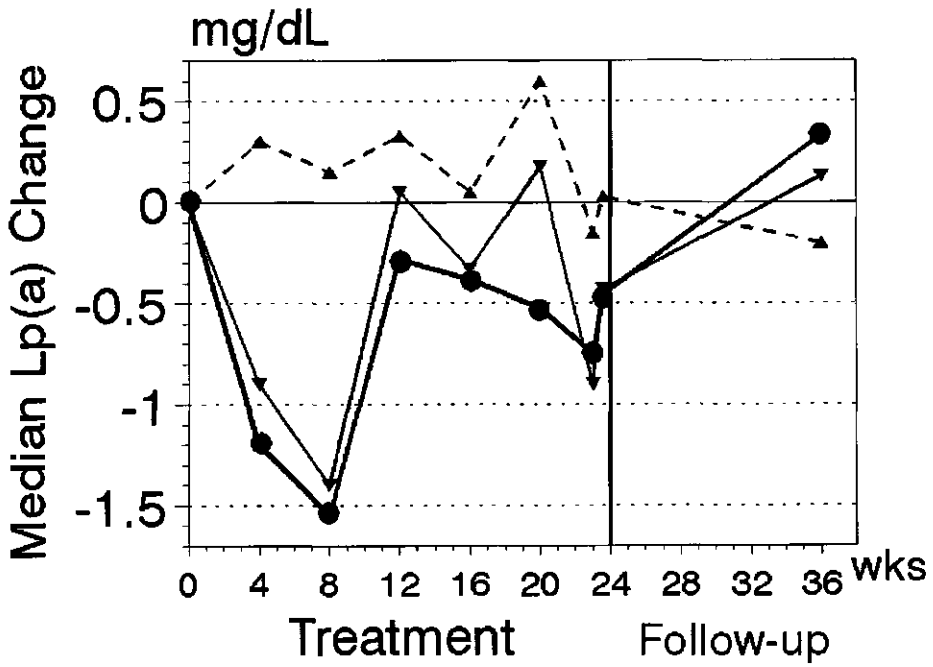

FIGURE 2. ***Trial A:*** **Median changes in serum levels of lipoprotein(a) [(Lp(a)] in subjects consuming five cups of cafetière (▼, n = 22) or filtered coffee (▲, n = 24) per day for 24 weeks, and the difference between the changes (●). No treatment was given in the follow-up period.**

Changes in Lp(a) were not associated with changes in other serum lipids and lipoproteins, or in liver enzymes ($P > 0.05$). In all four experiments, Lp(a) levels returned to baseline values after cessation of treatment.

We combined the individual data of the four trials and classified all subjects according to initial Lp(a) value (figure 3). On an absolute basis, individuals with high initial Lp(a) values appeared to benefit more from diterpene intake; the median fall of Lp(a) was -0.3 mg/dL in the lowest, -3.3 mg/dL in the middle, and -6.5 mg/dL in the upper tertile in subjects who received diterpenes, each relative to the median change in the corresponding tertile of controls.

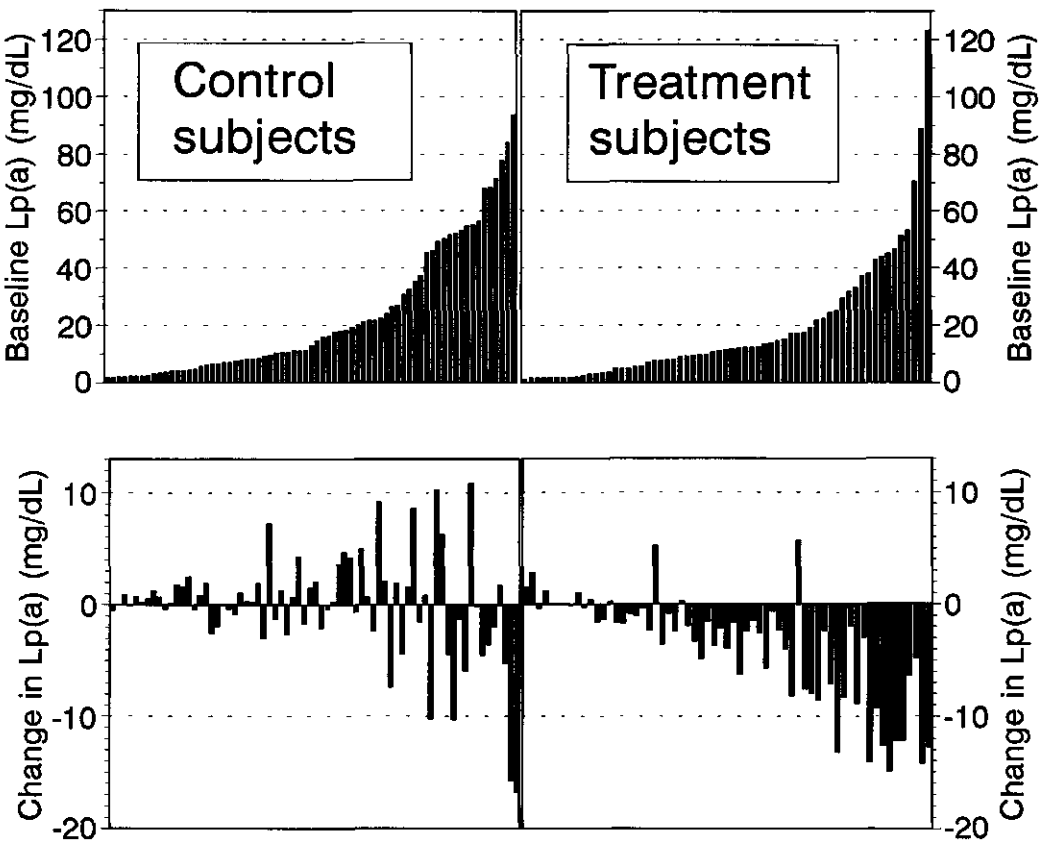

FIGURE 3. Individual baseline values (upper panels) and changes in serum lipoprotein(a) [Lp(a)] after four weeks (lower panels) in subjects who received placebo (control subjects, n = 71) or preparations rich in cafestol and kahweol (treatment subjects, n = 63) in four randomised, controlled trials.

TABLE 2. Effect of daily intake of preparations rich in coffee diterpenes for four weeks on serum lipoprotein(a) [Lp(a)] in four randomised, controlled trials. Subjects received filtered coffee (trial A) or placebo oil (trials B-D) in the run-in periods[a,b].

		No. of subjects	Diterpene content of preparation		Baseline level of Lp(a)		Lp(a) response after 4 weeks of treatment	
			Cafestol	*Kahweol*	*Median*	*Mean ± SD*	*Median*	*Mean ± SEM*
			mg/day	*mg/day*	*mg/dL*	*mg/dL*	*mg/dL*	*mg/dL*
Trial A	Filtered coffee	24	1	1	9.2	20.8 ± 22.3	0.3	0.2 ± 0.8
	Cafetière coffee	22	38	33	9.8	15.2 ± 19.9	-0.9	-2.0 ± 0.9*
Trial B	Placebo oil	16	0	0	17.2	25.9 ± 23.8	0.5	1.1 ± 0.9
	Coffee oil	16	85	103	14.9	29.1 ± 32.7	-4.8**	-5.5 ± 1.4**
Trial C	Placebo oil	15	0	0	17.7	24.4 ± 23.4	0.8	-1.0 ± 1.6
	Coffee oil	15	57	69	9.2	16.6 ± 16.6	-2.3*	-4.5 ± 1.3
	Coffee oil stripped of cafestol and kahweol	16	0	0	12.8	22.1 ± 25.5	-0.3	-1.1 ± 1.3
Trial D	Cafestol	10	63	1	11.5	13.9 ± 7.5	-3.5[c]	-3.5 ± 0.8[c]
	Cafestol plus Kahweol	10	61	51	11.5	13.9 ± 7.5	-3.1[c]	-3.9 ± 1.0[c]

[a] Changes were compared with Wilcoxon Rank Sum test and Mann Whitney U tests for medians, and with *t* tests for means.
[b] Response different from control group; * $P<0.05$, ** $P<0.01$.
[c] Response different from zero: $P<0.01$.

DISCUSSION

Preparations rich in the coffee diterpenes cafestol and kahweol reduced serum Lp(a) levels in four independent experiments. Females taking oral contraceptives were randomised over control and treatment groups, and none of the subjects took medications on a regular basis. Bias due to hormone intake or drug therapy [16] is thus unlikely. Furthermore, changes in dietary intakes, body mass, and alcohol use were minimal and similar for the control and treatment groups within each study [18,25,31]. Therefore, it is unlikely that the reductions in Lp(a) levels were due to anything else than treatment with coffee diterpenes.

Despite its structural similarity to LDL, the circulating level of Lp(a) is remarkably insensitive to dietary intervention; by now, only dietary *trans* fatty acids have consistently been proven to be effective, as they modestly raise Lp(a) levels [33-35]. Fish oils [36,37] as well as high doses of ascorbic acid [38] were found to reduce Lp(a) levels, but other attempts could not verify this [39-43]. Coffee diterpenes are thus among the few dietary components that modulate the circulating level of Lp(a).

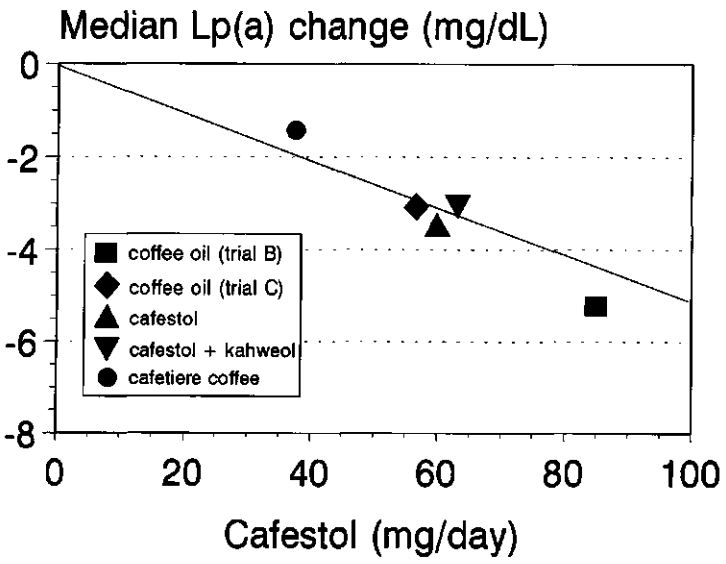

FIGURE 4. Relation of the observed median change in lipoprotein(a) [Lp(a)] after four weeks of treatment with the average cafestol intake per day (table 2) across the four trials. The responses are given relative to the median changes in the respective control groups.

Intake of cafestol alone was effective in reducing Lp(a) levels, and the addition of kahweol had little extra effect (trial D). Cafestol may thus be the sole Lp(a)-reducing principle in coffee oil. However, this trial was too small to fully exclude an additional effect of kahweol.

Health benefits

Individuals with high initial values of Lp(a) showed a larger drop in Lp(a) levels than those with low initial values (figure 3). The median fall across the four experiments depended on the daily amount of cafestol ingested; each 10 mg -- the amount present in two to three cups of cafetière coffee -- reduced Lp(a) levels by 0.5 mg/dL or 4% from baseline level after four weeks (figure 4). We would expect the same effect with Turkish and Scandinavian boiled coffee, as these types contain similar diterpene levels per cup as cafetière coffee. For espresso coffee, about seven cups per day are needed for a similar effect [32].

Could a lowering of Lp(a) levels with consumption of coffee diterpenes confer any health benefit? In a meta-analysis of eleven trials with preparations rich in cafestol and kahweol, we found that each 10 mg of cafestol ingested per day raises serum total cholesterol by 0.15 mmol/L, which was mostly due to an increase in LDL cholesterol (Chapter 8). The hypercholesterolaemic potency of coffee diterpenes thus overrules their potential beneficial impact on Lp(a) levels. This is evidenced by a higher rate of coronary heart disease in coffee drinkers in Norway [44], where boiled coffee is more common. Coffee diterpenes as such are thus unsuitable as a means of treatment for elevated Lp(a) levels, and a switch from filtered to unfiltered coffee is not warranted.

Short-term versus chronic intake

The present findings contradict our previous observation in a cross-sectional study that Norwegian boiled-coffee drinkers had *higher* Lp(a) levels than filter coffee drinkers [30]. The reason for this discrepancy is unknown. In our cross-sectional study and in each of the trials described here, serum samples of subjects who had ingested diterpenes and those of control subjects had been stored at -80 °C for a similar period with a maximum of one year. Therefore, even if sample

storing has affected Lp(a) levels, it is unlikely that it has affected the cross-sectional study and the experiments in opposite directions.

A second explanation could be the presence of some unknown confounding factor in our cross-sectional study, although the insensitivity of Lp(a) levels to environmental factors [16] argues against this possibility. Still, confounding by genetic differences cannot be fully excluded: all subjects were living in Southern Norway, but more people consuming boiled coffee may have descended from ancestors originating from the north, where boiled coffee is more common [27].

In trial A, Lp(a) levels were depressed by up to 15% in the first two months of intake of cafetière coffee, but the effect was strongly attenuated with prolonged use (figure 2). This indicates that the reducing effect of cafestol and kahweol may subside with prolonged intake. For other agents known to influence the serum level of Lp(a), this could have important implications. For instance, the evidence for the Lp(a)-elevating of dietary *trans* fatty acids is derived from short-term experiments only [33-35]. The present findings emphasize the need for intervention trials of longer duration in studying effects of diet or drugs on Lp(a) levels.

Mechanism

Short-term intake of coffee diterpenes raise serum levels of alanine aminotransferase [18,25,31,45,46], which may point at disturbed hepatocyte integrity [47]. The alterations in serum lipids and lipoprotein levels caused by coffee diterpenes may also be due to effects on liver cell metabolism. Apolipoprotein(a) is synthesised in the liver [48], and circulating levels of Lp(a) are largely determined by the rate of production [49]. Indeed, patients with biliary cirrhosis [50-52] or other liver diseases [51] have higher levels of lipids and liver aminotransferases, but had reduced plasma Lp(a) levels. It is thus possible that the reduction of Lp(a) by coffee diterpenes is also due to their effect on liver cell metabolism. Cross-sectional studies showed that chronic consumers of boiled [18] or espresso coffee [53] had no elevated levels of alanine aminotransferase. However, it remains speculative whether a transient effect on liver cell integrity may also result in an adaptation of Lp(a) metabolism in the human body.

Conclusions

Coffee diterpenes, which are present in unfiltered coffee brews, are among the few dietary constituents that modulate Lp(a) levels. An advise to switch from filtered to unfiltered coffee is not warranted, as cafestol and kahweol exert a range of other, negative health effects. The present findings also indicate that caution is needed in extrapolating results from short-term controlled trials to the chronic situation.

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8

the cholesterol-raising factor from coffee beans

review

ABSTRACT

Certain types of coffee brew raise the serum concentration of total and low-density lipoprotein cholesterol in humans, whereas others do not. The responsible factors are the diterpene lipids cafestol and kahweol, which make up about 1% (w:w) of coffee beans. Diterpenes are extracted by hot water, but are retained by a paper filter; this explains why filtered coffee does not affect cholesterol, whereas Scandinavian "boiled", cafetière, and Turkish coffee do. We describe the identification of the cholesterol-raising factors, their effects on blood levels of lipids and liver function enzymes, and their impact on public health, based on papers published up till December 1996 (ANNUAL REVIEW OF NUTRITION © 1997; 17: 305-24).

INTRODUCTION

Coffee and coronary heart disease risk

The relationship between coffee and coronary heart disease has long been controversial. In 1963, Paul et al [1] suggested for the first time that coffee drinking predisposes humans to myocardial infarction. This was not confirmed by epidemiologic studies that followed, although some did find a link [2]. One complicating factor was that coffee drinkers smoke more than abstainers [3]; indeed, smoking explained the original finding of Paul et al [4].

The uncertainties associated with epidemiologic analyses make it important to define the effects of coffee on risk factors for coronary heart disease in controlled experiments. Hypertensive effects may probably be disregarded, as the chronic impact of coffee or caffeine on blood pressure is small [2]. Attention should thus be focused on the effect of coffee drinking on serum cholesterol.

Coffee and serum cholesterol

Much of the information on coffee and cholesterol has come from Scandinavia, which has the highest coffee consumption world-wide [5]. In 1983, Thelle et al [6] found a strong association between coffee intake and serum cholesterol in Norway. They subsequently found in two experiments that withdrawal of coffee reduced cholesterol by 10% [7,8].

However, such an association was not consistently observed in the United States of America or western Europe [9]. The hypothesis was raised that the brewing method was critical; Scandinavians traditionally boil coffee grounds in water and pour the fluid into a cup without filtering it, whereas Americans and western Europeans mostly use a paper filter to separate the grounds from the brew [10]. Aro et al [11] showed in an experiment that "boiled" coffee indeed raised cholesterol, whereas in a parallel group filtered coffee had no effect. Bak & Grobbee [12] showed that actual boiling was not necessary: simply incubating the coffee grounds in water of 93 °C in a thermos jar produced the same effect. Later experiments showed that boiled coffee lost its entire cholesterol-raising potency when it was poured through a paper filter [13,14]. The brewing method thus made the crucial difference.

THE DITERPENES CAFESTOL AND KAHWEOL ARE THE CHOLESTEROL-RAISING FACTORS FROM COFFEE BEANS

The question now became which factor in boiled coffee affected cholesterol. While studying the content of solid coffee particles in boiled versus filtered coffee, Zock et al [15] fortuitously found that boiled coffee, upon centrifugation, displayed a thin floating layer of oil. Analyses confirmed that boiled coffee contains some 1 to 2 grams of lipid per liter, whereas filtered coffee contains hardly any [13]. Ingestion by ten volunteers of 1.3 g/day of such boiled-coffee lipids raised serum cholesterol by 23% [15]. Later studies with oils pressed directly from coffee beans produced similar effects [16-18]. Thus, the cholesterol-raising factor was a lipid.

Coffee oil largely consists of triglycerides -- which do not affect serum cholesterol when consumed in small amounts [19] -- but it also contains some 15% of diterpene esters of fatty acids [20]. Coffee oil that had been stripped of these diterpene esters no longer raised cholesterol in volunteers [16]. Heckers et al [21] then showed that ingestion of 148 mg of purified diterpene alcohols per day raised cholesterol by 32%, and similar rises were observed with purified diterpene esters [16,22].

The question now remained as to which diterpene was responsible. The

major coffee diterpenes are cafestol and kahweol. Pure cafestol can be made by hydrogenating a mixture of cafestol and kahweol isolated from coffee oil. However, it is difficult to purify kahweol. Urgert et al [22] therefore compared a supplement of pure cafestol with a mixture of cafestol and kahweol. Giving 63 mg of cafestol per day to ten volunteers raised cholesterol by 17%, whereas a mixture of 60 mg of cafestol plus 51 mg of kahweol per day only increased cholesterol by a further 2%. This suggested that the cholesterol-raising potential of a coffee brew depends mainly on its content of cafestol, and less on that of kahweol.

COFFEE DITERPENES RAISE LDL CHOLESTEROL AND TRIGLYCERIDES, AND ALSO AFFECT LIPOPROTEIN(a)

Experimental evidence

Eleven trials with humans given supplements of known diterpene content had been published by December 1996 (table 1). All subjects were healthy and normolipidaemic. For the present review, we performed a meta-analysis on these eleven trials, using as the independent variables the intakes of cafestol and kahweol per day, and as the dependent variables the mean changes in serum variables after four weeks of treatment.

CHOLESTEROL. In the combined trials, serum total cholesterol rose by 0.13 mmol/L (5.0 mg/dL) with each 10 mg of cafestol consumed per day and by 0.02 mmol/L (0.9 mg/dL) with each 10 mg of kahweol consumed per day for four weeks. This confirms that cafestol raises cholesterol more than kahweol does. The effect was linear up to 100 mg of cafestol per day (figure 1), the amount present in 15 to 30 cups of boiled coffee [23,24]. About 80% of the rise in total cholesterol was accounted for by low-density lipoprotein (LDL) cholesterol, and the rest was due to a rise in very-low-density (VLDL) cholesterol. High-density lipoprotein (HDL) cholesterol may fall slightly when cafestol and kahweol are ingested [15,16,22]. The pattern of changes induced by various diterpene-rich preparations in these eleven trials was in good agreement with that seen with boiled coffee [11-14,25].

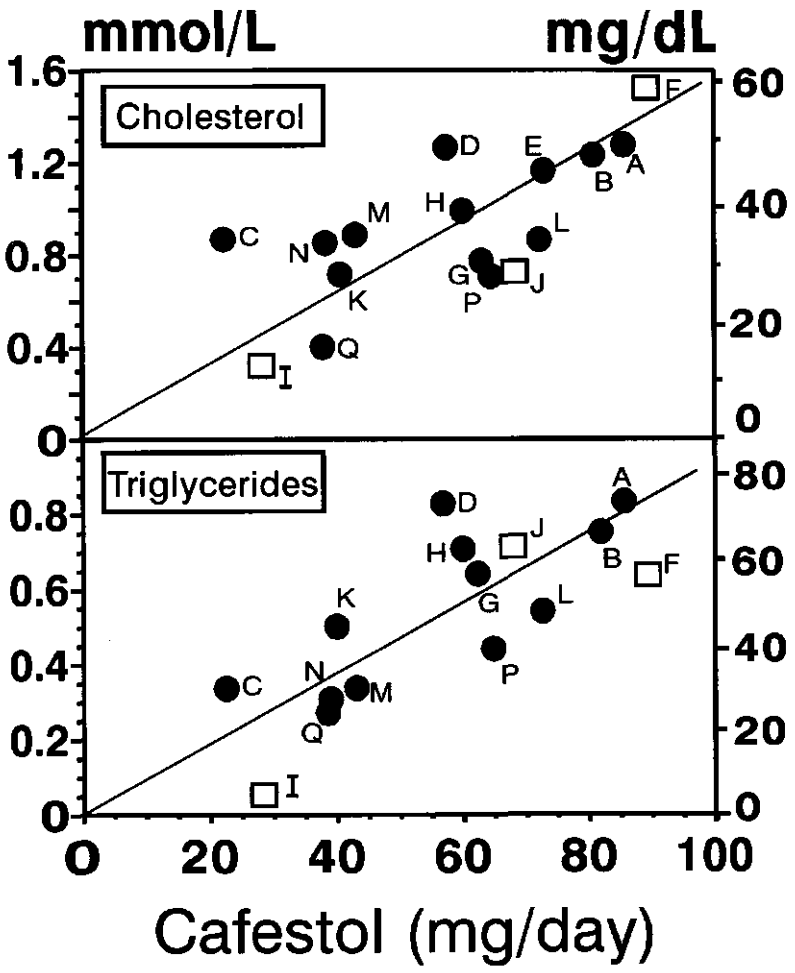

FIGURE 1. Relationship of daily cafestol intake with mean changes in cholesterol (upper panel) and triglyceride levels (lower panel) across eleven short-term experiments carried out by the Wageningen group (●), and others (□). Responses were adjusted for the mean changes in the concurrent control group, if present. Those for cholesterol were recalculated to the change after 4 weeks of treatment. Codes refer to treatment groups in different studies; A, coffee oil [16]; B, coffee oil enriched in non-triglyceride coffee lipids [16]; C, coffee oil depleted in non-triglyceride coffee lipids [16]; D, coffee oil [16]; E, mixture of pure diterpene esters [16]; F, mixture of pure diterpene alcohols [21]; G, pure cafestol esters [22]; H, pure cafestol plus kahweol esters [22]; I, Robusta oil [17]; J, Arabica oil [17]; K, Robusta oil [18]; L, Arabica oil [18]; M and N, coffee grounds [26]; P, lipid-rich extract from boiled coffee [15]; Q, cafetière coffee (values measured after 4 weeks of treatment) [35]. Least square best fit equations were: Δ Cholesterol (mmol/L) = 0.015 x cafestol (mg/day), and: Δ Triglycerides (mmol/L) = 0.009 x cafestol (mg/day).

TRIGLYCERIDES. Volunteers given boiled coffee [11-14,25] or preparations rich in coffee diterpenes [16-18,21,22,26] showed a marked rise in serum triglycerides. Again, cafestol was the major responsible factor; in ten volunteers, cafestol alone raised triglycerides by 86%, whereas addition of a similar amount of kahweol to the treatment further increased the response by only 7% [22].

Our regression analyses of eleven trials produced the same result; triglycerides rose by 0.08 mmol/L (7.3 mg/dL) with each 10 mg of cafestol per day and by 0.01 mmol/L (1.2 mg/dL) with each 10 mg of kahweol per day for two to six weeks (figure 1). However, most of the rise in triglycerides may subside with chronic intake of coffee diterpenes (see below).

LIPOPROTEIN(a). Lipoprotein(a), which consists of an LDL-particle attached to apolipoprotein(a), is a risk factor for cardiovascular diseases [27]. *Trans* fatty acids are the only dietary compounds that consistently affect lipoprotein(a) [28-30]. Coffee diterpenes also proved effective; in four of the trials done by the Wageningen group, each 10 mg of cafestol (plus kahweol) per day reduced serum lipoprotein(a) by 0.5 mg/dL or 4% after four weeks (Chapter 7). However, there was a marked disparity between short- and long-term intake of diterpenes (see below).

Epidemiologic evidence

Observational studies in Norway [16,31,32], Finland [33], and Sweden [34] that compared chronic users of boiled coffee to those who used filtered coffee provide insight into effects of chronic exposure to coffee diterpenes.

CHOLESTEROL. In the five observational studies, serum cholesterol was raised on average by 5% with chronic intake of each five cups of boiled coffee per day. Controlled trials with unfiltered coffee yielded an estimate of 6.8% per five cups (figure 2). Therefore, the effect as measured in epidemiologic studies may have been slightly attenuated by measurement errors. However, a partial return of serum cholesterol to baseline values was observed in a long-term experiment with 0.9 liter of cafetière coffee per day; serum cholesterol was raised by 10% after three months of intake, but the effect was reduced to 6% after six months of intake

[35]. Therefore, chronic intake of cafestol permanently raises cholesterol, but trials lasting fewer than three months may slightly overestimate the effect.

TRIGLYCERIDES. Stensvold et al [32] found a negative association between intake of boiled coffee and serum triglycerides in Norwegians. An even stronger negative trend was observed for filtered coffee, leaving a positive net effect of 7% per five cups of boiled coffee. A similar difference between boiled- and filtered-coffee drinkers was found by Weusten-van der Wouw et al [16]. In controlled trials, rises in triglycerides of up to 22% per five cups of boiled or cafetière coffee were found (figure 2), which suggests that most of the effect is transient with chronic intake. Indeed, 0.9 liter of cafetière coffee per day raised triglycerides by 26% in the first month, but the effect had fallen to only 7% after six months of intake [35].

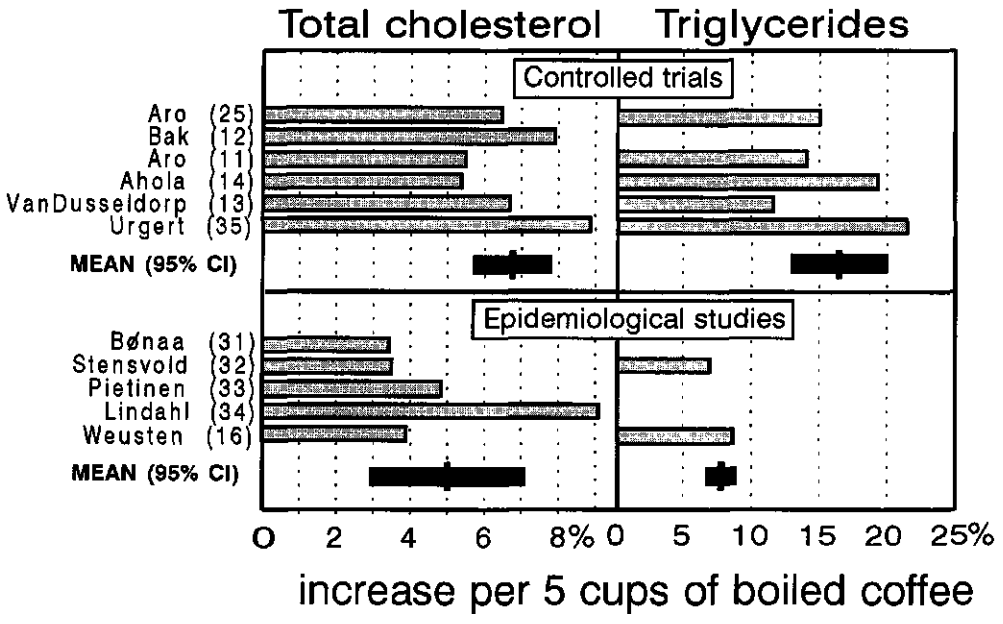

FIGURE 2. Percentage increases in serum cholesterol and triglyceride levels with each five cups of boiled or cafetière coffee consumed per day in controlled trials (upper panels), and percentage differences with each five cups consumed per day between consumers of boiled versus filtered coffee in epidemiologic studies (lower panels). Black bars indicate the 95% Confidence Interval of the mean response per five cups.

TABLE 1. Summary of experiments in which supplements of known coffee diterpene content were given to volunteers[a].

Investigators	Design	Test period	No. of subjects	Treatment	Dosage		Serum responses		
					Cafestol	Kahweol	Cholesterol	Triglyceride	ALT[b]
		weeks	*n*		*mg/d*	*mg/d*	*mmol/L*	*mmol/L*	*U/L*
Zock et al [15]	Before/after	6	10	Fat from boiled coffee	64[c]	56[c]	1.06**	0.51**	10**
Weusten-van der Wouw et al [16]	Parallel	4	16	Placebo oil	0	0	-0.04	0.00	1
			16	Coffee oil	85	103	1.23**	0.83**	41**
			15	Coffee oil rich in non-triglycerides	81	98	1.19**	0.75**	47**
			16	Coffee oil poor in non-triglycerides	22	26	0.83**	0.34**	18**
	Parallel	4	16	Placebo oil	0	0	-0.06	-0.02	4
			12	Coffee oil	57	69	1.21**	0.81**	38**
			15	Coffee oil without diterpenes	0	0	0.04	-0.02	0
	Before/after	6	3	Pure cafestol + kahweol	73	58	1.71*	1.83	31
Heckers et al [21]	Parallel	4	5	Placebo	0	0	-0.23	-0.05	--
			5	Pure cafestol + kahweol	89	59	1.24[d]	0.55[d]	--
Urgert et al [26]	Before/after	3	15	Coffee grounds	43	55	0.67**	0.34**	13**
	Parallel	3	7	Placebo	0	0	0.01	0.06	3
			7	Coffee grounds	39	49	0.66**	0.36*	21*
Mensink et al [18]	Cross-over	3	5	Robusta oil	40	2	0.53	0.49**	13
			6	Arabica oil	72	53	0.65*	0.54**	17*
Van Rooij et al [17]	Parallel	6	12	Placebo oil	0	0	0.07	0.06	-4
			12	Robusta oil	30	1	0.52	0.14	9
			12	Arabica oil	70	87	1.14**	0.81**	64**
Urgert et al [22]	Cross-over	4	10	Pure cafestol	63	1	0.79**	0.65**	18**
				Pure cafestol + kahweol	60	51	0.94**	0.71**	46**
Urgert et al [35]	Parallel	24	24	Filtered coffee	1	1	-0.05	0.13	-1
			23	Cafetiere coffee	38	33	0.29*	0.19	8**

[a] Response different from the control group in parallel studies, or different from zero in cross-over studies and studies with a before/after design: * $P<0.05$, ** $p<0.01$

[b] In case of different assays for ALT, activities were recalculated by multiplying the response by the ratio of the reported upper limit of normal (ULN) to our upper limit or normal.

[c] Estimated from a total amount of diterpenes of 120 mg

[d] No statistical testing provided

LIPOPROTEIN(a). Only one epidemiologic study has examined the effect of coffee type on serum lipoprotein(a); in 150 Norwegian boiled-coffee drinkers, the median level of lipoprotein(a) was 65% higher than in 159 matched filtered coffee drinkers [36]. This contradicts the lipoprotein(a)-reducing capacity of diterpenes observed in clinical trials. Although a chance finding cannot be excluded, it may imply that coffee diterpenes only reduce lipoprotein(a) levels in the short run.

In summary, most of the rises in total and LDL cholesterol caused by coffee diterpenes persist with chronic intake, whereas most of the rise in triglycerides subsides. Coffee diterpenes reduce serum lipoprotein(a) in the first months of intake only. These observations have implications for experiments on the relationship between diet and lipoproteins in general; caution is needed in extrapolating results from studies lasting weeks or even months to chronic intakes, and corroborative evidence should always be sought in long-term trials or epidemiologic observations.

HUMANS ARE MORE SENSITIVE TO COFFEE DITERPENES THAN ANIMALS

The mechanism by which coffee diterpenes affect lipid metabolism is largely unknown. The group of Drevon in Norway studied the involvement of the LDL receptor, which is located on cell membranes and is responsible for the removal of LDL cholesterol from the bloodstream. Cafestol indeed decreased the uptake of LDL cholesterol into human fibroblasts [37] and hepatoma cells [38], but raised it in an intestinal cell line [39]. More studies are needed to clarify this discrepancy.

The effects of cafestol and kahweol seem to be unique to *Homo sapiens* (figure 3). The same batch of coffee oil that raised cholesterol in humans [16] produced no effect in Cebus or Rhesus monkeys in two different laboratories [40] (figure 3). Sanders & Sandaradura [41] did report that Syrian hamsters responded to boiled coffee, but an attempt to replicate this was unsuccessful [42], as were other studies with hamsters [43,44]. Diterpenes raised cholesterol in only one [45] out of three [42,45,46] studies with Wistar rats, and no effect was found in gerbils [43] or rabbits [47]. The absence of effect could not be explained by differences in dosage, mode of administration, treatment duration, or cholesterol content of the

background diet. One may speculate that differences in absorption or metabolism of coffee diterpenes account for this marked species specificity. The negative results in a range of animal species emphasize the need to rely on human data.

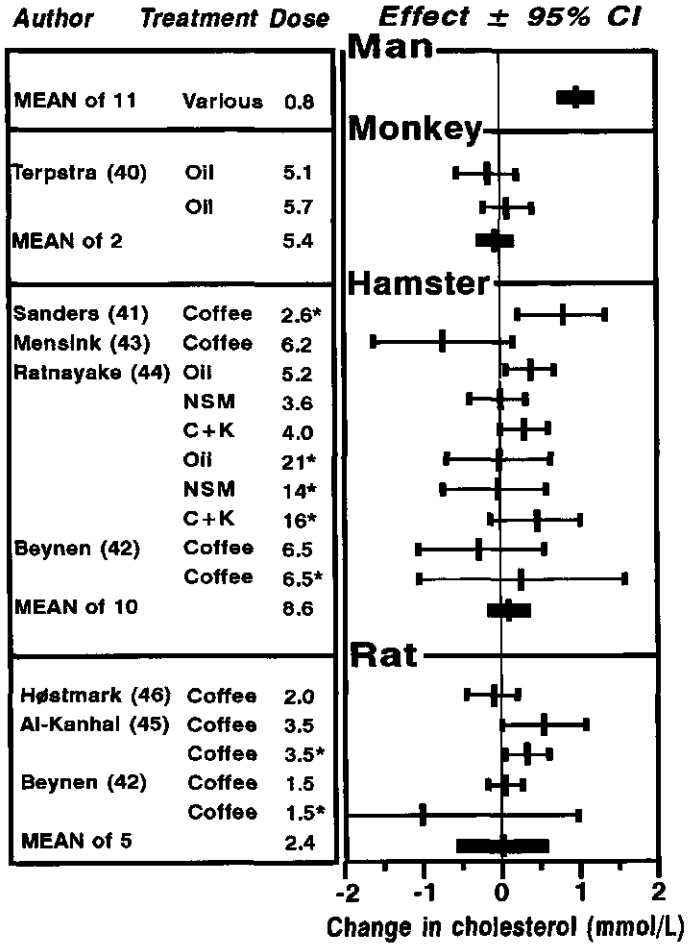

FIGURE 3. Comparison of the effect of cafestol in man with that in various animal species. Bars represent the 95% Confidence Intervals of the treatment effects. Treatment periods varied from 4 to 20 weeks; oil = coffee oil; coffee = boiled coffee; NSM = non-triglyceride lipid fraction from coffee beans; C+K = purified cafestol and kahweol. Dose is expressed as mg per day of cafestol per kg of body weight, and high fat or high cholesterol background diets are indicated by '*'. The value for man represents the overall mean change in cholesterol in eleven experiments in which preparations of known diterpene content were given (cf Figure 1).

THE LIVER IS THE TARGET ORGAN FOR COFFEE DITERPENES

Intake of coffee diterpenes [16,17,26] or unfiltered coffee [16,35] raised the serum activity of alanine aminotransferase (ALT, formerly SGPT) in volunteers. In a pooled analysis of 147 volunteers who received diterpenes in our own trials, each 10 mg of cafestol or kahweol per day raised alanine aminotransferase by 2 to 3 U/L, or 8 to 12% (Chapter 9). Aspartate aminotransferase (AST, formerly SGOT) usually also rose, but less [16,17,22,26,35].

A rise of liver enzyme activity in serum may indicate injury to hepatocytes [48]. This is not due to cholestasis, as coffee diterpenes reduce rather than raise the serum activity of γ-glutamyltransferase and alkaline phosphatase [16,22,26,35]. It is unlikely that a perturbation of liver cell function explains the effects of coffee diterpenes on blood lipids, because both cafestol and kahweol raise aminotransferases, but kahweol has little effect on blood lipids [22].

DITERPENE LEVELS IN COMMERCIAL ROAST AND GROUND COFFEES

The main commercial species of coffee are arabica and robusta coffee. Robustas contain no kahweol and less cafestol than arabicas [20]; intake levels of diterpenes may thus be reduced by increasing the proportion of robustas in coffee blends. However, in the US and western Europe consumers prefer the taste of arabicas [5]. As a result, diterpene levels in commercial roast and ground coffees in these countries are fairly constant at 400 to 500 mg each of cafestol and kahweol per 100 g of grounds [23]. Diterpene levels in coffee beans are not affected by decaffeination, and are affected little by roasting [23,49].

In addition to cafestol, robustas also contain some 16-*O*-methylcafestol [50]; the effects of this diterpene in man are unknown. Its levels are negligible in blends with a high proportion of arabicas [23], and five cups of unfiltered coffee brewed with pure robusta grounds will provide only 2 to 3 mg of 16-*O*-methylcafestol. This is probably too low to substantially affect blood lipids.

DITERPENE LEVELS IN COFFEE DEPEND ON BREWING TECHNIQUE

Extraction of diterpenes into coffee brew

About 10% of the diterpenes present in the grounds used to make unfiltered coffee ends up in the brew, either in oil droplets or in small bean particles [26]. In volunteers who consumed oily solutions of diterpenes, recovery of coffee diterpenes from faeces was about 5%, compared with 25% in subjects who consumed coffee grounds [51]. This suggested that most of the diterpenes ingested from the grounds are absorbed. Indeed, intake of 8 g dry weight of coffee grounds containing 39 to 55 mg of each diterpene per day for three weeks raised serum cholesterol by 0.65 mmol/L in volunteers [26]. Diterpene analyses of coffee brews should thus include the contribution of grounds floating in the brew. In addition, frequent ingestion of coffee beans or of grounds with turbid coffee brews should be avoided.

Brews with low levels of diterpenes

FILTERED COFFEE. Diterpenes do not pass through a paper filter (table 2), which explains why controlled trials have shown no impact of filtered coffee on blood lipids [12-14,52]. Fried et al [53] found that 720 mL of filtered coffee per day did raise serum cholesterol by 0.25 mmol/L after four weeks, relative to no coffee. However, no rise was seen in the groups drinking 360 mL of regular coffee per day, or 720 mL of decaffeinated coffee. Therefore, the effect seen might be due to chance.

INSTANT COFFEE. Instant (or soluble) coffee is consumed world-wide [5]. It is almost devoid of cafestol and kahweol (table 2). Burr et al [54] found that five cups of instant coffee per day raised serum cholesterol by 0.12 mmol/L in volunteers. No rise was observed in other trials of instant coffee [55-58], and its effect on blood lipids -- if any -- should be small.

PERCOLATED COFFEE. Percolated coffee was popular in the US until a decade ago. Although boiled and unfiltered, it is poor in diterpenes (table 2). In the percolator pot, the brew is constantly recirculated through a bed of grounds, which likely functions as a filter cake retaining the lipids. Whatever the mechanism,

predicted effects of percolated coffee on blood lipids are minimal, although this has not yet been verified in human experiments.

TABLE 2. Reported levels of coffee diterpenes in various coffee brews, and the estimated effect on serum cholesterol of consumption of five cups of coffee per day[a].

	Ratnayake et al [66]	Urgert et al [23]		Gross et al [24]		MEAN		Effect on serum cholesterol with five cups/day[b]
	C or K[c]	C	K	C	K	C	K	
Coffee type	*mg/cup*	*mg/cup*		*mg/cup*		*mg/cup*		*mmol/L*
Paper-filtered	0.1	0.1	0.1	<0.1	<0.1	0.1	0.1	<0.01
Instant	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.02
Percolator	--	0.3	0.3	--	--	0.3	0.3	0.02
Mocha	--	1.1	1.4	2.3	2.3	1.7	1.9	0.13
Espresso	3.6	1.5	1.8	1.0	1.0	2.0	2.1	0.15
Cafetière	1.6	3.5	4.4	--	--	2.6	3.0	0.20
Turkish	3.4	3.9	3.9	5.3	5.4	4.2	4.2	0.32
Boiled	8.4	3.0	3.9	7.2	7.2	6.2	6.5	0.47

[a] Cup sizes are 150 mL for filtered, instant, cafetière, and boiled coffees, 60 mL for Turkish and mocha coffees, and 25 mL for espresso coffees

[b] Estimations are based on the assumption that each 10 mg/day of cafestol consumed raises cholesterol by 0.13 mmol/L, and each 10 mg/day of kahweol raises it by 0.02 mmol/L (cf Figure 2)

[c] Ratnayake et al [66] reported total diterpene content; values are calculated assuming that levels of kahweol and cafestol were equal

Brews with moderate levels of diterpenes

MOCHA COFFEE. Mocha coffee is common in Italy and Spain [5]. Based on diterpene contents, each five cups of mocha per day should raise serum cholesterol by 0.13 mmol/L (5.4 mg/dL) (table 2, figure 1). Observational studies in Italy [59-61] and Spain [62] are compatible with a cholesterol-raising effect of mocha,

but experiments [63-65] have failed to confirm this. These studies may have suffered from insufficient power, due to short treatment periods [63] and low dosages [64,65]. However, a major lipid-raising effect of mocha coffee consumption appears excluded.

ESPRESSO COFFEE. The concentration of diterpenes per 100 mL of espresso is high [23,66]. However, in Italy, espresso coffee is served in quantities as small as 25 mL [67], and absolute levels per cup are moderate (table 2). D'Amicis et al [65] found that three cups of espresso per day non-significantly raised total cholesterol by 0.10 mmol/L and LDL cholesterol by 0.15 mmol/L, relative to tea. This is consistent with a moderate effect of espresso on serum cholesterol.

Espresso coffees sampled in Italy provided more cafestol per cup than those from other countries [23]. This is partly explained by the ratio of grounds to water, which is highest in Italian espresso and is a major determinant of diterpene levels in unfiltered coffee [23]. In view of the increasing popularity of espresso world-wide [10], future studies should address other factors that may affect diterpene levels, such as brewing device, contact time of steam with grounds, and mesh width of the filter grid.

Brews with high levels of diterpenes

CAFETIÈRE COFFEE. Cafetière coffee -- known as French press coffee in the US -- is fairly uncommon, though its popularity is increasing [10]. Based on its diterpene content, five cups per day are estimated to raise serum cholesterol by 0.13 to 0.27 mmol/L (table 2, figure 1), The cholesterol-raising potential of cafetière coffee was confirmed in a controlled trial; 0.9 liter of strong cafetière coffee per day raised cholesterol by up to 0.52 mmol/L [35].

TURKISH COFFEE. Turkish coffee is consumed in Greece, the former Yugoslavia, Turkey, and the Middle-East including Israel ("mud" coffee), and by Muslims in various countries. Five cups provide 21 mg of each diterpene, which with daily consumption will raise serum cholesterol by 0.32 mmol/L (13 mg/dL) (table 2, figure 1). However, diterpene levels fluctuate strongly with the amount of grounds floating in the brew, and levels of up to 50 mg of each diterpene per five cups have been reported [23]. Studies in Israel [68-70] and in Serbia [71] have indeed shown an association of cholesterol with coffee intake, but the effects of

Turkish coffee have not yet been examined experimentally. Its diterpene content suggest that it may be as least as effective in raising cholesterol as is boiled coffee.

BOILED COFFEE. Differences in the ratio of coffee grounds used per liter of water may explain the large differences in reported diterpene levels of Scandinavian boiled coffee (table 2). On average, five cups per day are estimated to raise cholesterol levels by 0.47 mmol/L; this is in agreement with results of experimental [11-14,25,72] and epidemiologic studies [16,31-34].

Decaffeinated coffee brews

Superko et al [52] found in a large trial that decaffeinated filtered coffee raised LDL cholesterol by 0.23 mmol/L compared with regular coffee. Diterpene levels of beans are unaffected by decaffeination [23,49]. Therefore, to explain the effect of decaffeinated filtered coffee as seen by Superko et al [52], one has to assume that besides diterpenes there is a second cholesterol-raising factor in coffee beans, which is somehow introduced or activated by decaffeination, and which passes through a paper filter. We find this unlikely, especially in view of the negative results of other trials of decaffeinated filtered coffee [53,73,74]. For lack of a better explanation we ascribe the finding of Superko et al [52] to chance, though the large number of subjects argues against that.

PUBLIC HEALTH CONSIDERATIONS

Risk of coronary heart disease

Filtered, instant, and percolated coffee contain negligible levels of diterpenes (table 2). The switch from percolated to filtered coffee in the US thus appears not to have contributed to the fall in cholesterol levels over the last decades [75]. The low intake of brews other than filtered and instant coffee in western Europe also excludes a major association with blood lipids in these countries. Little is known about effects of coffee or caffeine intake on other risk factors for cardiovascular

diseases, such as oxidisability of LDL particles, vascular proliferation, or thrombosis. However, prospective cohort studies from the US and western Europe mostly failed to find a link between coffee intake and cardiovascular disease [76-78], and a major effect of filtered, percolated, and instant coffee seems unlikely.

In the US, about 15% of coffee consumed is decaffeinated [5], and those who use decaffeinated coffee often do so because of health concerns [79,80]. In a study of 46,000 health professionals in the United States, Grobbee et al [81] actually found a 63% higher risk of coronary mortality in consumers of decaffeinated coffee compared with coffee abstainers. As this association was not seen in a cohort of 86,000 US women studied by Willett et al [82], there is no consistent evidence that filtered decaffeinated coffee raises coronary risk. However, there is no evidence for a protective effect either; the major proven benefit of decaffeinated coffee is that it does not interfere with falling asleep.

Boiled coffee used to be the dominant type in Scandinavia, but nowadays more than three-quarters of Scandinavians use filtered coffee. This switch in brewing practices is thought to explain one-third [83] to one-half [2] of the 10% fall in serum cholesterol in Scandinavia since 1970, and to have contributed significantly to the concurrent fall in coronary mortality [84,85]. This was supported by results of the National Health Screening Service in Norway, which examined a population largely consisting of boiled-coffee drinkers. Over the period from 1980 to 1986, a relative risk for coronary mortality of 3.3 was found in heavy coffee drinkers versus abstainers [86]. The risk was reduced to 1.4 after six more years of follow-up [87], which may be due to a change in brewing practices. Thus, experimental and epidemiologic studies both suggest that a high intake of boiled coffee raises the risk of dying from coronary heart disease.

No prospective studies have examined the effects of other unfiltered brews. Because of their moderate amounts of cafestol and kahweol, mocha and espresso coffee appear harmless with consumption of a few cups per day. Turkish and cafetière coffee are rich in diterpenes, and a recommendation to limit their use in favour of filtered or instant coffee seems justified in patients with a high cholesterol level or an increased coronary risk.

Risk of liver disease

Could the perturbation of liver cells by diterpene ingestion as suggested by rises in serum alanine aminotransferase activity affect hepatic health in consumers of unfiltered coffee? Alanine aminotransferase activity remained raised during half a year of daily intake of cafetière coffee [35], but it was not raised in life-long consumers of boiled [16] or espresso coffee [88]. Mortality rates of liver cirrhosis have been typically low in Scandinavian countries and appear to have been unaffected by the nation-wide switch to filtered coffee [89]. Also, habitual coffee drinkers have lower serum γ-glutamyltransferase levels than non-drinkers have [88,90-93] -- a finding that might have a bearing on the association of coffee use with a reduction in the risk of alcoholic liver cirrhosis [94,95]. The negative relationship with γ-glutamyltransferase was stronger for boiled than for filtered coffee [92]. Therefore, clinically relevant damage to liver cells in healthy subjects drinking unfiltered coffee appears unlikely. However, subclinical hepatic injury cannot be excluded at the present time, and patients with elevated alanine aminotransferase levels would do well not to drink more than a few cups of boiled, Turkish, *or cafetière coffee per* day.

CONCLUSIONS

Coffee beans and some types of coffee brew -- though not filtered or instant coffee -- contain the diterpenes cafestol and kahweol. They are not removed by decaffeination. Cafestol, and to a lesser extent kahweol, raises serum total and LDL cholesterol in humans. Patients at increased risk of heart disease should thus be advised to select brews low in diterpenes. Triglycerides also rise with cafestol intake, but the effect could be transient with chronic intake. Both cafestol and kahweol appear to affect the integrity of liver cells, as suggested by a modest rise of alanine aminotransferase activity in serum. All effects are reversible after withdrawal of the diterpenes.

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9

concluding remarks

In the general introduction (Chapter 1), the objective of the studies described in the present thesis was subdivided into five research questions. This chapter gives the answers to these questions, recommendations for further research, and the main conclusions.

Cafestol and kahweol are available from coffee grounds

Intake of coffee grounds strongly raised serum levels of lipids and alanine aminotransferase (Chapter 2). The rises were similar to those observed with the same amount of diterpenes in oils (Chapter 8). Up to 90 percent of diterpenes in unfiltered coffee may be carried by floating grounds; these will thus contribute substantially to the hyperlipidaemic and hepatocellular effects of unfiltered coffee.

The brewing method is a main determinant of diterpene content

Diterpene contents differed strongly between coffee types; boiled, cafetière, and Turkish coffee contained 3 to 4 mg, mocha and espresso coffee 1 to 2 mg, and filtered, percolated, and instant coffee less than 0.2 mg of cafestol or kahweol per cup. Diterpene contents also varied 'within' a particular type of coffee; ten-fold differences between samples were found for Turkish, boiled, and espresso coffee. Analyses in coffee prepared under laboratory conditions showed that for espresso and boiled coffee most of the variation could be ascribed to the amount of grounds used per litre of water, whereas amounts in Turkish coffee strongly depended on the amount of floating grounds in the brew. Effects of brewing time were minimal. Diterpene contents in beans were not affected by roasting or decaffeination.

Cafestol is more hyperlipidaemic than kahweol, but both raise alanine aminotransferase levels

In a cross-over study with ten volunteers, intake of cafestol alone strongly raised serum levels of lipids and of alanine aminotransferase. Addition of kahweol to the supplement had little effect on serum lipids, but doubled the responses in aminotransferases (Chapter 4). The study involved only men so as to exclude possible variation due to gender. However, a meta-analysis of eleven human trials,

most of which also included females, confirmed a higher lipid-raising capacity of cafestol (Chapter 8). Consequently, our conclusion that cafestol is the prime cholesterol-raising factor in unfiltered coffee probably applies to both sexes. Both cafestol and kahweol increased serum liver aminotransferases, which indicates that lipid metabolism and liver cell integrity may be affected by coffee diterpenes through different pathways.

Prolonged intake of coffee diterpenes persistently raises serum levels of alanine aminotransferase

Volunteers who had consumed five to six cups of strong cafetière coffee per day for half a year had higher serum levels of alanine aminotransferase than those who had consumed filtered coffee (Chapter 5). Chronic consumers of unfiltered coffee did not have elevated levels of alanine aminotransferase [1,2]. We cannot exclude that a longer intake is needed to allow alanine aminotransferase levels to return to baseline values. Cafetière coffee also raised serum lipids; after half a year of intake, levels of total and LDL cholesterol were still raised, but the rise in triglycerides had almost disappeared (Chapter 5). The lipidaemic effects agree with those observed in epidemiological studies of consumers of boiled versus filtered coffee (Chapter 8).

We prescribed strong coffee; each 150 mL cup of cafetière coffee provided 6 mg of cafestol. Cafetière coffee sampled from consumers at large provided about half this amount per cup (Chapter 2). The linear relation between cafestol and changes in lipid levels (Chapter 8) implies that the expected effects with such moderately strong brews are about half the effects observed in this study.

Coffee diterpenes affect levels of lipoprotein(a)

Norwegians with a chronic high intake of boiled coffee had higher serum lipoprotein(a) levels than peers drinking filtered coffee (Chapter 6). However, intake of preparations rich in coffee diterpenes reduced Lp(a) levels in four different experiments in volunteers who were not accustomed to drinking unfiltered coffee (Chapter 7). We could not offer a likely explanation for the divergence between short and long term exposure to coffee diterpenes.

HETEROGENEITY AND MAGNITUDE OF THE RESPONSES

Nine human trials with preparations rich in coffee diterpenes, including those described in this thesis, were done at our department between 1990 and 1996. The trials involved 147 subjects who received preparations rich in diterpenes and 78 control subjects. We pooled their individual responses to obtain more insight into the heterogeneity and the magnitude of the effects.

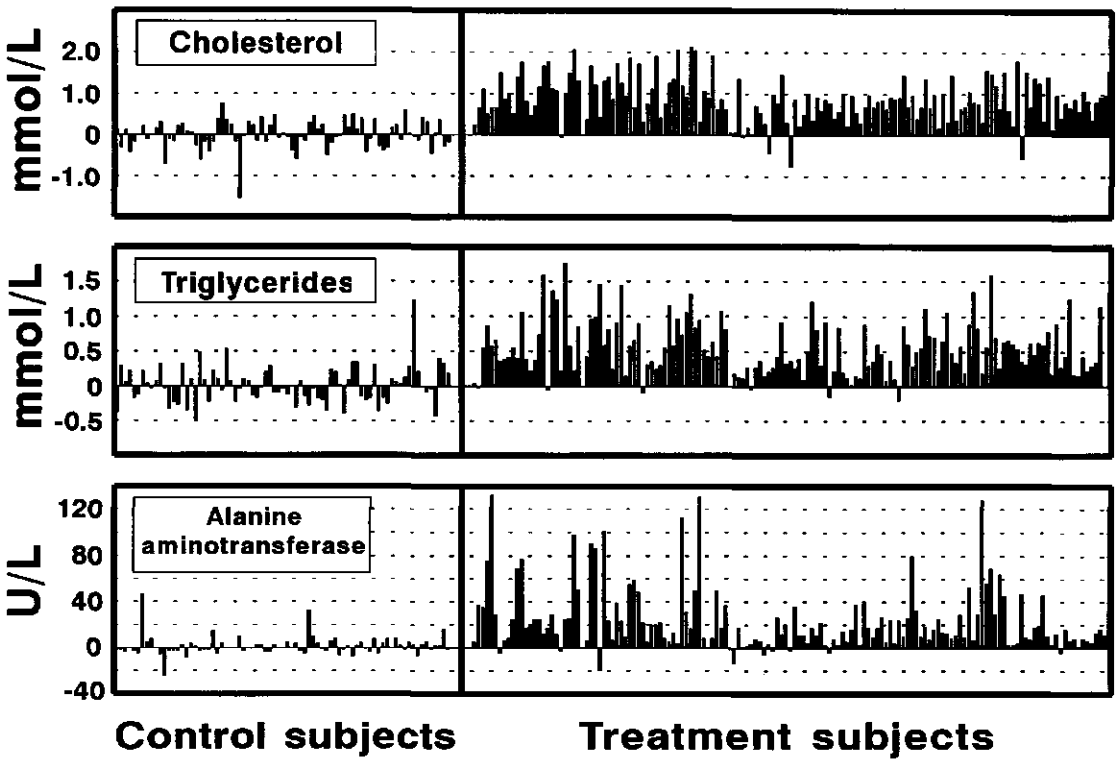

FIGURE 1. Individual responses from baseline in serum levels of total cholesterol, triglycerides, and alanine aminotransferase in volunteers receiving placebo or no treatment (control subjects, n = 78) or volunteers receiving supplements containing 22-85 mg/day of cafestol and 0-103 mg/day of kahweol (treatment subjects, n = 147) in any of the nine experiments carried out at our department.

Blood lipids and lipoproteins

Levels of total cholesterol rose in 142 and triglycerides in 141 of the 147 subjects who received diterpenes in any of the trials (figure 1). Thus, the vast majority of subjects were susceptible to the impact of coffee diterpenes on lipid metabolism. We also calculated the responses standardised for the cafestol dose; each 10 mg of cafestol ingested per day for four weeks raised total cholesterol by 0.17 mmol/L and triglycerides by 0.11 mmol/L (figure 2). Gender appeared to explain some of the variation in responses; responses of males (n = 85) were higher than those of females (n = 62) by 16 percent for total cholesterol, 30 percent for LDL cholesterol, and 20 percent for triglycerides (unpublished observations).

About 75 percent of the rise in total cholesterol is due to a rise in LDL cholesterol (figure 2). The effect is substantial; each 10 mg of cafestol per day raises LDL cholesterol similar to an increase in intake of about 12 g per day of saturated [3] or *trans* fatty acids (Peter Zock, personal communication). The effect on LDL cholesterol persists with chronic intake of cafestol (Chapters 5 and 8), and is the principal reason for coffee consumers with a high risk of coronary heart disease to restrict their intake of unfiltered coffee.

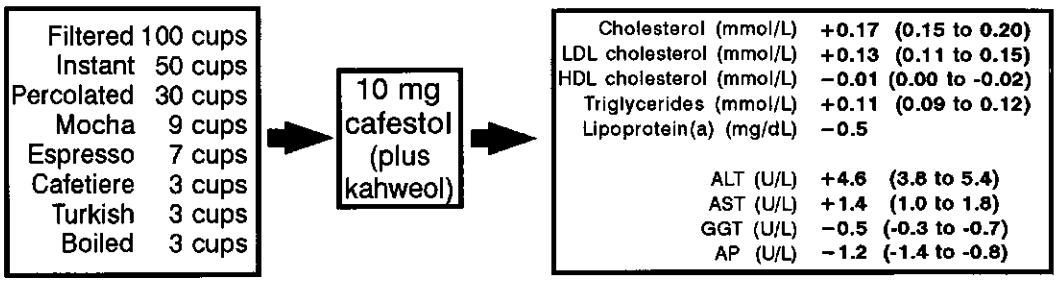

FIGURE 2. Effects of each 10 mg per day of cafestol (plus kahweol) ingested from different types of coffee for four weeks on serum lipids and lipoproteins, alanine (ALT) and aspartate aminotransferase (AST), γ-glutamyltransferase (GGT), and alkaline phosphatase (AP). The estimates are based on diterpene analyses in coffee brews (Chapter 2), and the mean responses (95% confidence interval) in 147 volunteers receiving preparations rich in diterpenes in any of the nine experiments carried out at our department. The value for Lp(a) represents the median change in 63 subjects (Chapter 7).

The remainder of the rise in total cholesterol levels is due to a rise in VLDL-cholesterol, as indicated by the increase in triglyceride levels (figure 2). As most of this effect appeared transitory (Chapters 5 and 8), the impact of drinking unfiltered coffee on triglyceride levels is of minor importance for atherogenic risk.

The remarkable impact of cafestol on lipid metabolism is emphasised by their effect on serum levels of lipoprotein(a); each 10 mg per day of cafestol reduced Lp(a) levels by 0.5 mg/dL or four percent from baseline levels. Due to the unfavourable effects of diterpenes on LDL cholesterol and alanine aminotransferase levels and the attenuation of the effect on Lp(a) levels in the long run, advising individuals with high Lp(a) levels to drink unfiltered coffee is not warranted.

Blood liver enzymes

The effects of coffee diterpenes on the liver were evaluated by their effects on four enzymes: alanine and aspartate aminotransferase, γ-glutamyltransferase, and alkaline phosphatase. Most notable were changes in alanine aminotransferase; diterpene treatment raised alanine aminotransferase level in 137 of 147 subjects (figure 1). Again, there was a striking effect of gender; responses were 64 percent higher in males than in females (unpublished observations).

The rise in alanine aminotransferase persisted during half a year of consumption of unfiltered coffee (Chapter 5). However, the changes are not dramatic; each 10 mg of cafestol plus kahweol raises alanine aminotransferase similarly to an increase in daily alcohol intake of about 3 drinks, or in body mass index of about 1 kg/m^2 [4]. The changes in other liver enzymes are probably negligible (figure 2), and, as was true of alanine aminotransferase, all changes were reversible after cessation of diterpene intake. It is therefore most unlikely that intake of coffee diterpenes causes chronic damage to liver cells or an impairment of liver function. However, the mechanism by which coffee diterpenes raise alanine aminotransferase levels is unknown. Subclinical injury to hepatocytes, such as small-scale fatty infiltration of the liver or slightly increased breakage of liver cell membranes, cannot be excluded; the large differences in individual susceptibility suggests that if these processes occur, they may be less innocuous in some individuals. Therefore, subjects with raised alanine aminotransferase levels might also do well to restrict their intake of unfiltered coffee.

gone unrecognised.

Changes in synthesis or clearance of lipoproteins by the liver are likely explanations for the impact of cafestol on lipid metabolism [23]. None of the animals tested so far responded to coffee diterpenes, but it may prove worthwhile to test animals in which essential genes for cholesterol metabolism have been modified. One experiment in humans indicated that serum lathosterol levels which are an indicator of cholesterol synthesis, were not markedly raised by boiled coffee [24]. Such findings are not definitive, and should be corroborated by turnover studies with labelled cholesterol precursors or labelled lipoproteins.

We recently found that cafestol and kahweol raise the serum activity of cholesterylester transfer protein (unpublished observations). This enzyme catalyses the transfer of cholesterylesters from HDL to particles with lower density (LDL and VLDL), and increased activity is thus consistent with the changes in lipid levels caused by cafestol. However, it is not clear whether a rise in cholesterylester transfer protein is a cause or a consequence of rises in LDL and VLDL cholesterol [25]. More research is needed to clarify the relevance of these findings.

Finally, lipidaemic effects caused by treatment with androgenic or other steroids partly resemble those caused by cafestol [26]. Steroid treatment also reduces Lp(a) levels [27], and may affect liver cells [28]. It is of interest to study whether cafestol exerts its action through pathways related to steroid metabolism in humans, and whether this could be related to its stronger effects in men than in women.

Epidemiological studies

Observational studies in heavy consumers of unfiltered coffee might help to explain some of the discrepancies in effects of short-term versus chronic intake of coffee diterpenes, notably those on alanine aminotransferase and lipoprotein(a). In epidemiological studies that address lipidaemic effects of diet in general, the method of coffee brewing should be recorded and, if possible, also cup size and brewing strength should be taken into account. In the Netherlands, this may particularly be meaningful if the study population includes a fair number of consumers who drink most of their coffee in retail outlets (where a substantial part of the coffee served is a type of espresso), or of Muslim immigrants.

Experimental studies

Experiments should focus on factors that may explain the heterogeneity of responses in lipids and liver enzymes, such as sex or genotype (e.g. for genes encoding for apolipoproteins or lipid transfer enzymes). The use of milk in coffee is also of interest; cafetière-coffee drinkers (Chapter 5) who used milk had larger increases in serum lipids and alanine aminotransferase than those who consumed black coffee (unpublished observations). This hypothesis needs to be tested specifically in an experiment.

A biochemical marker for cafestol intake may be useful, both for experiments (to monitor compliance) and for epidemiological studies (to measure exposure). Measurement of cafestol conjugates in urine proved modestly successful [29], and more attempts to measure cafestol in serum should be undertaken.

Analytical studies

Due to the large variation in diterpene content in different samples of the same type of coffee, more insight is needed into factors that determine intake levels of diterpenes with unfiltered brews that are consumed by large populations. This recommendation particularly holds for Turkish coffee (e.g. study of the actual amount of particles that is consumed with the brew), and espresso coffee (e.g. examination of the influence of brewing characteristics, such as steam pressure and mesh width of the filter grid).

CONCLUSIONS AND IMPLICATIONS

Daily intake of the coffee diterpene cafestol and -- to a lesser extent -- of kahweol strongly and persistently raises blood levels of total and LDL cholesterol. Cafestol occurs in high amounts in boiled, Turkish, and cafetière coffee, and in moderate amounts in espresso and mocha coffee; a chronic high intake of these types of coffee will increase the risk of coronary heart disease. Amounts of cafestol in filtered and instant coffee are too low to affect blood levels of LDL cholesterol.

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29. Urgert R, Kosmeijer-Schuil TG, Katan MB. Intake levels, sites of action and excretion routes of the cholesterol-elevating diterpenes from coffee beans in humans. *Biochem Soc Transact* 1996;24:800-806.

summary

Consumption of Scandinavian boiled coffee -- prepared by boiling coarsely ground coffee beans in water and decanting the fluid without the use of a filter -- increases the blood concentration of low-density lipoprotein cholesterol and triglycerides in humans. The disturbance of lipid metabolism is attributable to the diterpenes cafestol and kahweol, which are natural constituents of coffee beans. Cafestol en kahweol do not pass through a paper filter, which explains why regular drip-filtered coffee does not affect blood lipids and lipoproteins. Coffee diterpenes also raise the blood concentration of alanine aminotransferase. This enzyme occurs in high concentrations in the liver, from where it leaks in small amounts into the bloodstream. Elevations of alanine aminotransferase in the bloodstream may thus point at a disturbed integrity of liver cells. It is unknown whether the effects of cafestol and kahweol on lipid metabolism are related to their effects on liver cell metabolism.

The objective of the present thesis was to further specify the effects of drinking unfiltered coffee on health. In our studies, we focused on determinants of diterpene concentrations in coffee prepared with various brewing techniques, and on effects of cafestol and kahweol on blood concentrations of lipids and liver enzymes in humans.

In unfiltered coffee brews, diterpenes are present in oil droplets and in floating bean particles or 'grounds'. The latter may carry up to 90 percent of the diterpenes in so-called 'turbid' coffee brews, such as Turkish or Middle-Eastern coffee. Coffee diterpenes raise cholesterol concentrations when they are consumed dissolved in oil, but their availability from grounds was unknown. We fed coffee grounds to volunteers in two separate experiments, and observed marked rises in blood concentrations of lipids and alanine aminotransferase. We concluded that floating grounds contribute significantly to the effects of unfiltered coffee on blood lipid and liver-enzyme concentrations. Therefore, frequent ingestion of floating bean particles or of coffee beans should be avoided (CHAPTER 2).

Many studies had shown that life-long consumers of boiled coffee have raised blood concentrations of cholesterol. However, one study showed that Norwegians who were life-long consumers of boiled coffee did not have higher alanine aminotransferase concentrations than matched filter coffee drinkers. A possible explanation is that alanine aminotransferase concentrations return to baseline concentrations with prolonged intake of cafestol and kahweol. We examined this hypothesis in a randomised trial with 46 volunteers drinking 0.9 litre of either cafetière or filtered coffee for 24 weeks. Cafetière coffee raised alanine aminotransferase by up to 80 percent over baseline relative to filtered coffee. After six months of treatment, the rise was still 45 percent. The effect thus did not fully subside with prolonged intake of cafetière coffee. In one out of three subjects drinking cafetière coffee, alanine aminotransferase concentrations exceeded the upper limit of normal, and in one out of seven subjects, concentrations exceeded twice the upper limit of normal; high intakes of coffee brews rich in cafestol and kahweol may therefore be responsible for unexplained elevations of this enzyme in apparently healthy individuals. Patients with (a history of) liver disease or patients with otherwise elevated concentrations of alanine aminotransferase might do well in limiting their intake of coffee rich in diterpenes. Cafetière coffee also strongly raised the blood concentrations of total and low-density lipoprotein cholesterol, and those of triglycerides; most of the rises in total and low-density lipoprotein cholesterol persisted, but most of the rise in triglycerides was transitory within the treatment period of six months (CHAPTER 5).

We also studied the effects of unfiltered coffee and of supplements rich in coffee diterpenes on blood concentrations of lipoprotein(a), which is a risk indicator of cardiovascular diseases. The lipoprotein(a) particle consists of a low-density lipoprotein particle attached to a large glycoprotein, but despite the structural similarity between lipoprotein(a) and low-density lipoprotein, lipoprotein(a) concentrations are highly insensitive to dietary influences. We found in an observational study that the median concentration of blood lipoprotein(a) in 150 Norwegians habitually consuming boiled coffee was 65 percent higher than in 159 peers drinking filter coffee (CHAPTER 6). However, in four trials with preparations rich in cafestol and kahweol, each 10 mg/day of cafestol ingested for four weeks actually reduced lipoprotein(a) concentrations by four percent from baseline values (CHAPTER 7). Coffee diterpenes are thus among the few dietary components known

to affect blood lipoprotein(a) concentrations. We can as yet offer no likely explanation for the discrepancy between the effects with short-term versus chronic intake. The reducing effect on lipoprotein(a) was diminished, but not reversed, within half a year of consumption of cafetière coffee. Obviously, short-term treatment with coffee diterpenes in individuals with high lipoprotein(a) concentrations is not warranted: any potential benefit of a reduction of lipoprotein(a) concentrations by cafestol and kahweol is overshadowed by their effects on total and low-density lipoprotein cholesterol.

Persistent effects of coffee diterpenes on lipid metabolism thus appear to be confined to increases in total and low-density lipoprotein cholesterol. In a meta-analysis of eleven human trials with supplements rich in coffee diterpenes, we found that each 10 mg of cafestol (plus kahweol) per day predicts a rise in blood cholesterol concentrations of 0.15 mmol/L. Coupled to our analyses of diterpenes in coffee brews as described in Chapter 2, this prediction implies that chronic intake of five cups per day of boiled, Turkish, or cafetière coffee will raise blood cholesterol 0.22 to 0.29 mmol/L, and five cups of mocha and espresso will raise blood cholesterol by 0.08 to 0.11 mmol/L. Effects of filtered, instant, and percolated coffee on cholesterol concentrations are negligible (CHAPTER 8).

In conclusion, daily consumption of brews rich in cafestol -- boiled, cafetière, and Turkish coffee, and to a lesser extent also espresso and mocha coffee -- increases the risk of coronary heart disease due to a persistent increase in the blood concentration of low-density lipoprotein cholesterol. Prolonged intake of large amounts of unfiltered coffee also modestly raises alanine aminotransferase concentrations. Patients with an increased risk of heart disease or with an elevated concentration of alanine aminotransferase should be advised to select brews low in cafestol -- filtered, instant, and percolated coffee.

samenvatting

'Gekookte' koffie wordt gezet door koffiemaalsel op te koken met water en het brouwsel af te gieten zonder te filtreren. Deze koffiedrank was tot voor kort geliefd in Scandinavische landen. Mensen die gekookte koffie drinken hebben een verhoogd bloedgehalte van cholesterol en van triglyceriden. Beide bloedlipiden verhogen het risico op het krijgen van hartziekten. De stoffen uit gekookte koffie die verantwoordelijk zijn, zijn de diterpenen cafestol en kahweol. Ze zitten van nature in koffiebonen, en komen bij het koffiezetten in de drank terecht. Een papieren filter houdt de koffiediterpenen tegen, en daarom verhoogt filterkoffie het bloedlipidengehalte niet. Hoe cafestol en kahweol de bloedlipiden verhogen is niet bekend. Misschien speelt de lever een rol: cafestol en kahweol verhogen het gehalte van het enzym alanine aminotransferase in het bloed. Dit kan duiden op een ongunstig effect op levercellen.

De gezondheidseffecten van cafestol en kahweol staan centraal in dit proefschrift. We hebben de hoeveelheid cafestol en kahweol in koffiedranken gemeten, en onderzocht hoe de bloedgehaltes van lipiden en leverenzymen veranderen bij gezonde proefpersonen die cafestol en kahweol binnenkrijgen.

Cafestol en kahweol komen alleen voor in ongefilterde koffie. Hierin zijn ze verdeeld over oliedruppeltjes en zwevende koffiedeeltjes. We wisten al dat het consumeren van olie met koffiediterpenen verhogend werkt op het bloedgehalte van lipiden en van alanine aminotransferase. Of ze ook geabsorbeerd worden uit koffiedeeltjes was nog niet bekend. Daarom hebben we in twee experimenten koffiedrab gegeven aan gezonde vrijwilligers (HOOFDSTUK 2). In beide experimenten deed de koffiedrab de bloedgehalten van lipiden en van alanine aminotransferase flink stijgen. Diterpenen in zwevende koffiedeeltjes dragen dus bij aan het cholesterol-verhogende effect van ongefilterde koffie.

Vervolgens hebben we de hoeveelheid cafestol en kahweol in koffiedranken bepaald (HOOFDSTUK 3). Turkse koffie en cafetière koffie bleken even veel diterpenen te bevatten als gekookte koffie: gemiddeld zo'n 3 tot 4 milligram van elk diterpeen per kop. Turkse koffie en cafetière koffie zouden dus het cholesterol even sterk moeten verhogen als gekookte koffie. Espresso bevatte minder

diterpenen, 1 tot 2 milligram per kop, doordat espresso meestal uit kleine kopjes gedronken wordt. Gefilterde koffie en oploskoffie bleken vrijwel geen diterpenen te bevatten. De manier waarop de koffie wordt bereid is dus van groot belang. Hoeveel iedere koffiesoort het cholesterol verhoogt, staat verderop in deze samenvatting beschreven. Cafeïne-vrije koffiebonen bleken net zoveel diterpenen te bevatten als 'gewone' koffiebonen: ongefilterde koffie die gezet is met gedecafeïneerd maalsel zal dus ook het cholesterol verhogen.

De vraag bleef of het cafestol of kahweol is die het cholesterol verhoogt. Deze kennis is nuttig om het werkingsmechanisme te onderzoeken. We wilden daarom in een experiment de effecten van cafestol vergelijken met die van kahweol. Zuivere cafestol hadden we, maar het lukte ons niet om zuivere kahweol te verkrijgen. Daarom hebben we cafestol vergeleken met een mengsel waarin naast cafestol ook kahweol zat (HOOFDSTUK 4). We vonden dat cafestol het gehalte van cholesterol en van triglyceriden sterk verhoogde. Echter, het mengsel liet praktisch dezelfde resultaten zien. Dit betekent dat het cholesterol-verhogende effect van ongefilterde koffie voornamelijk veroorzaakt wordt door het gehalte aan cafestol. De resultaten van het leverenzym alanine aminotransferase waren heel anders: cafestol verhoogde dit enzym, maar het mengsel verhoogde het nog veel meer. Omdat kahweol dus niet het cholesterolgehalte maar wel het gehalte van alanine aminotransferase verhoogt, concluderen we dat koffiediterpenen de bloedlipiden en de levercellen op verschillende manieren beïnvloeden.

Mensen die hun hele leven al gekookte koffie drinken hebben gemiddeld een hoger cholesterolgehalte dan mensen die filterkoffie drinken. Het gehalte van het leverenzym alanine aminotransferase bleek echter niet verhoogd in kookkoffiedrinkers. Het effect op de levercellen zou dus van voorbijgaande aard kunnen zijn. We hebben een experiment gedaan om te onderzoeken of dit inderdaad zo is (HOOFDSTUK 5). Stevige koffiedrinkers werden verdeeld over twee groepen: de ene groep dronk een half jaar lang zes mokken sterke cafetière koffie per dag, de andere groep dronk evenveel filterkoffie. In de cafetière drinkers steeg inderdaad het gehalte van alanine aminotransferase, maar na een half jaar cafetière koffie was het nog steeds verhoogd. Dit geeft aan dat het effect van cafestol en kahweol op de levercellen niet binnen een half jaar verdwijnt. Deze kennis is bruikbaar in de medische praktijk: als dit enzym verhoogd is bij ogenschijnlijk gezonde mensen, zou het kunnen dat ze veel ongefilterde koffie drinken. In dit experiment vonden we ook

dat cafetière koffie het bloedgehalte van cholesterol en triglyceriden verhoogde. Na een half jaar bleek de stijging in cholesterol vrijwel onveranderd, maar was een groot deel van de stijging in triglyceriden verdwenen. Een hoger risico op hartziekten met het chronisch drinken van ongefilterde koffie wordt dus voornamelijk veroorzaakt door het effect op cholesterol.

Ook hebben we het effect van koffiediterpenen op het bloedgehalte van lipoproteine(a) bestudeerd. Een hoog lipoproteine(a) gehalte is in verband gebracht met een hogere kans op hart- en vaatziekten. De hoeveelheid lipoproteine(a) die in het bloed circuleert is echter voor een groot deel erfelijk bepaald, en is ongevoelig voor stoffen uit de voeding. Mensen die doorgaans veel gekookte koffie drinken bleken wel hogere waarden van dit lipoproteine te hebben dan filterkoffie-drinkers (HOOFDSTUK 6). Vreemd genoeg bleek uit vier andere studies dat inneming van cafestol en kahweol gedurende een korte periode juist het lipoproteine(a) verlaagt (HOOFDSTUK 7). We hebben hier geen logische verklaring voor. Het is wel duidelijk dat het geen zin heeft om mensen die een hoog lipoproteine(a) gehalte hebben te behandelen met koffiediterpenen: de nadelige effecten op het cholesterolgehalte zouden een eventueel positief effect op lipoproteine(a) teniet doen.

In HOOFDSTUK 8 hebben we de huidige gegevens over de cholesterolverhogende diterpenen gecombineerd. Iedere kop Turkse, gekookte, of cafetière koffie die per dag gedronken wordt, verhoogt het cholesterolgehalte met ongeveer één procent. Voor espresso geldt dat ieder kopje per dag het cholesterol verhoogt met een derde tot een half procent. Filterkoffie en oploskoffie verhogen het cholesterolgehalte niet.

Conclusie

De diterpenen cafestol en kahweol zijn aanwezig in gekookte, Turkse, en cafetière koffie, en in mindere mate in espresso. Filter- en oploskoffie bevatten slechts sporen cafestol en kahweol. Cafestol en kahweol verhogen het cholesterolgehalte van het bloed zolang ze dagelijks worden geconsumeerd. Bovendien verhogen ze het bloedgehalte van het leverenzym alanine aminotransferase. Stevige koffiedrinkers en mensen met een verhoogd risico op hartziekten of een leveraandoening doen er verstandig aan de consumptie van diterpeenrijke koffie te beperken.

wintervertelling

Het was guur en bijtend koud. De stadsklokken hadden pas vier uur geslagen, maar toch was het al helemaal donker. De oude Urge liep driftig heen en weer in zijn kantoor. Hij rangschikte een stapeltje papieren, borg die behoedzaam op in een map, en schreef er met grote letters *MIJN PROEFSCHRIFT* op. 'Af!' riep hij triomfantelijk. Hij had niet gemerkt dat achter hem iemand het kantoor had betreden. Het was de zwaargeleerde Zock, een man met weinig haar maar met een hoop publicaties. 'Is het af?' vroeg deze. Urge knikte en glunderde: 'Zeventien februari lever ik het in'. 'Mooi,' sprak Zock, 'dan nu alleen nog een dankwoord!' 'Een wat?' riep Urge. Zijn voldoening sloeg om in ergernis. 'Een dánkwoord? Waar is dat in hemelsnaam goed voor? En vertel me eens, beste Zock,' zei hij smalend, 'wie zou ik dan wel moeten bedanken?' De zwaargeleerde was even aangeslagen. 'Nou ja,' stamelde hij, 'iedereen die een steentje heeft bijgedragen aan de totstandko...' 'Humbug!' schreeuwde Urge. 'HUMBUG!' Luid mopperend beende hij het kantoor uit.

Die avond kon Urge de slaap niet vatten. 'Een dankwoord', gromde hij. 'Wat een onzin! Zonder mij was er van dat hele onderzoek geen bliksem terechtgekomen.' Opeens sloeg de slaapkamerdeur met een klap open. In de deuropening stond een doorschijnende gestalte. 'Wat moet dat?' riep Urge. 'Zeg, hoe haal je het in je hoofd?' Langzaam kwam de gestalte dichterbij. 'Wel potverdomme!' schreeuwde Urge. Plots zag hij dat de ongewenste gast een bordje om zijn nek had hangen. 'Geest van het verleden,' las Urge hardop. 'Kijk eens aan,' hoonde hij, 'een geest. Geesten bestaan niet! Donder op!' De doorschijnende figuur was hem nu tot op een halve meter genaderd en strekte zijn armen uit. In een vloeiende beweging pakte hij Urge die zwaar tegenstribbelde bij de kraag van zijn nachthemd, en trok hem door het vensterraam mee de nacht in. 'Hé!' riep Urge met overslaande stem. 'Zet me neer! Waar gaan we naar toe?' 'Zwijg!' sprak de geest. Zijn strenge toon bezorgde Urge kippevel. Even later stonden ze in een schaars verlicht vertrek. Aan een bureau zat een bebaarde man te puffen en te zweten, terwijl hij met een dikke rode stift lange strepen over een bundeltje paperassen trok. 'He, dat is Martijn!',

riep Urge verrast, 'maar wat ziet hij er slecht uit...' 'Logisch', sprak de geest, 'hij zit net een versie van jouw eerste artikel te lezen.' Urge moest even slikken. 'Jij denkt dat je het alleen hebt gedaan,' zei de geest, 'maar zonder Martijn was jij niet ver gekomen, mannetje. En wat te denken van professor Hautvast. Zonder zijn talenten was de vakgroep er niet eens geweest. Laat staan je proefschrift!' 'Maar ze worden daar toch voor betaald?' sputterde Urge tegen. 'Mag ik nu weer naar bed?' 'Nee! Het begint pas!' snauwde de geest. 'Want wat te denken van Guido en Robert? Truus en Peter? Marga? Waar zou jij zijn zonder hun toewijding en hun technische vernuft? Jij kan nog niet eens een pipet en een staafmixer uit elkaar houden! En laten we het eens hebben over al de mensen die voor jou koffiedrab en pillen en liters koffie hebben weggewerkt? Of over Joanna en Hedwig die de medische keuring hebben gedaan? Over Joke en Geke en Jan en Paul van het priklab?' Urge was bleek weggetrokken. Hij stopte zijn vingers in zijn oren en riep met hese stem: 'Humbug, humbug, humbug!' 'Oh ja?' gilde de geest, 'heb je nog wel eens gedacht aan Angela dan, die jou die uitgevallen vriezer heeft doen vergeten? En aan Arjan die dozen met poep voor jou vervoerde? Jolieke die koffie voor je verzamelde? Susan die je taalfouten moest verbeteren?' De geest wist van geen ophouden meer. 'En al je kamergenoten dan? Nicole Z, Gerda, Toine. Daar was je toch zo dol op? En zo kan ik nog wel even doorgaan. Menrike, Karin, Paul, Trudie. Zonder hen was het een stuk minder gezellig geweest! Oh ja, nu we het toch over gezelligheid hebben...' De geest sleurde Urge mee naar een keuken waar vijf studenten ontbijt stonden klaar te maken voor deelnemers. 'Hier, kijk dan toch!' riep hij woedend. 'Maar geest,' jammerde Urge, 'boterhammen smeren, dat kan toch iedereen?' 'Oooooh, wat ben jij onnozel!'. De geest raakte nu volledig door het dolle heen: 'Natasja. Maud. Marjan. Mariska. Henny. Doorzetters zijn het, Urge. Cracks! En jij praat over boterhammen smeren? Je mag in je handjes klappen met zulke studenten. Jij... jij.... egocentrische kl...!' Plotseling stopte de geest. Op zachte toon vervolgde hij: 'En dan zwijg ik nog over Saskia. Man, man. Wat die allemaal heeft gedaan om die proeven mogelijk te maken....' Hij slaakte een diepe zucht. Urge zweeg. In zijn hart begon hij zich af te vragen wat hij eigenlijk wel zelf had gedaan. 'Ik wil naar bed,' zei hij schor. Hij had het nog niet gezegd of hij lag alweer onder de vertrouwde dekens.

Maar niet voor lang. 'Meekomen, Mr Ego!' donderde het plots door de kamer, 'ik ben de geest van het heden!' Urge begreep dat ieder verzet zinloos was. Voor hij het wist, waren ze bij een drukker in Middelburg. Achter een grote tafel zat een jongen op honderden verschillende manieren een koffieboon op een kaft te plakken. 'Ja', stamelde Urge, 'ik weet het al. Dat is mijn broertje Marcel en die heeft veel werk aan de voorkant van mijn proefschrift gehad...'. 'Inderdaad', riep de geest van het heden. 'Weet je nog? Eind januari riep je dat je de kaft gewoon blauw zou maken, begin februari had je al twintig ontwerpen van deze arme jongen ontvangen! En wat te denken van je maatjes Peter en Annet die je straks ook nog eens belangeloos als paranimf terzijde staan? Of wou je soms alléén voor de leeuwen? En alle andere mensen die jouw verblijf op de vakgroep zo plezierig maken, zoals Baukje en Martine en Robert en Riekie en Marie en Riet? Nou?' En weg was hij weer. Even abrupt als hij gekomen was...

Opnieuw hoefde Urge niet lang te wachten. Daar verscheen de geest van de toekomst al in de deuropening. Met een lange en knokige wijsvinger gebaarde hij Urge hem te volgen. Met afhangende schouders slofte hij achter de geest aan. Plotseling waren ze in een herberg. Aan een tafeltje zaten zijn vrienden van The Gang, Marianne, Mark, een groepje toneelspelers, Maarten, Jacco, Francesca. Urge ving delen van het gesprek op. 'Pfff, wat is het eigenlijk een verwaande kwast'. 'Hij heeft zich lelijk in de kaart laten kijken, en dat zal-ie weten ook!' 'Van mij mag-ie lekker van zichzelf genieten, jongens, ik zal hem daar niet meer bij storen! Kom, het leven gaat door, we némen er nog een!' 'Over wie hebben ze het?', vroeg Urge aan de geest. Deze zweeg hardnekkig en gebaarde enkel hem weer te volgen. Ze kwamen aan bij een groot huis. Het smalle voortuintje stond vol fietsen. 'He geest', riep Urge blij, 'daar woon ik!'. Ze betraden het pand. In de keuken zaten zijn huisgenoten en zijn vrouw Caroline uitgeblust rond de tafel. Een hele poos was het stil. Toen sprak Caroline bedroefd: 'Het is gewoon een hufter, en dat is het!' Er werd instemmend gemompeld. 'Ach ach', zei Urge, 'dat moet wel weer over een hele nare man gaan. Hebben ze het over dezelfde persoon?' De geest sloeg geen acht op zijn vraag en leidde hem weer naar buiten. Een poos gingen ze zwijgzaam voort door de kille nacht, tot ze bij een groot hek kwamen dat toegang gaf tot een verlaten park. Bij het openen knarsten de scharnieren als hadden ze in een

eeuwigheid niet bewogen. Flarden mist hingen tussen de toppen van de kale bomen. Het was doodstil. 'Waar zijn we in vredesnaam?' huiverde Urge. Op een bankje zat een oude man, gehuld in een versleten deken. Roerloos staarde hij naar een stapel witte boekjes bij zijn voeten. 'Wat een ontzettend zielige en eenzame man,' zei Urge. Vertel mij, geest, wie is deze stakker?' Waarom zit hij niet thuis bij het haardvuur? Waarom heft hij geen drinkgelag aan met zijn vrienden? Waarom zit hij hier moederziel alleen in de kou?' De geest zweeg onverbiddelijk en wees naar de boekjes. Urge boog zich voorover om beter te kijken. Op de voorkant van ieder boekje prijkte een koffieboon. 'NEE!,' riep Urge ontsteld uit. 'Dat kan niet! Dat wil ik niet! Geest, ik verzeker je, ik ben niet meer de man die ik was. Ik weet nu wat ik te danken heb aan mijn vrienden, mijn familie, mijn collega's'. Smekend wierp hij zich voor de voeten van de geest. 'Beloof me dat ik nog een kans krijg... Zeg me dat er nog hoop is, zeg het me! In godsnaam!' Maar toen Urge opkeek, was hij alleen. 'Neeee!' kreunde hij. In de verte kraste een kraai.

Urge ontwaakte door het gekwetter van vogeltjes. Snel keek hij om zich heen. Hij zag geen hek. Geen bomen. Alleen een bed. Zijn eigen bed. Hij sprong op en rende naar de kalender. Het was de ochtend van de zeventiende februari. 'Het is nog niet te laat,' stamelde hij dankbaar. Hij gooide het raam open, stak zijn hoofd naar buiten en jubelde luidkeels: 'Het is nog niet te laat!'. Opgelucht zette hij zich achter zijn tafel, pakte een vel papier en een pen, en begon te schrijven: *Velen hebben een steentje bijgedragen aan de totstandkoming van dit proefschrift. Graag wil ik al deze mensen hartelijk bedanken...* Het papier schitterde in het zonlicht dat naar binnen scheen. Het leek wel lente.

about the author

Rob Urgert was born in The Hague, the Netherlands, on July 9, 1968. He passed secondary school, gymnasium, at the Goese Lyceum in Goes in 1986. That same year he started the study 'Human Nutrition' at the Wageningen Agricultural University. During this study he worked for seven months at the Tanzanian Food & Nutrition Centre in Dar es Salaam, Tanzania. He obtained his MSc-degree in Human Nutrition in 1992 with main topics in food chemistry, toxicology, and epidemiology. In September 1992, he started working as a research assistant at the Department of Human Nutrition within the SENECA elderly study. In October 1992 he was appointed as a PhD-student (OIO) at the Department of Human Nutrition, where he started the research which resulted in the present thesis. He attended the Annual New England Epidemiology Summer Program at Tufts University, Boston, USA in the summer of 1993. He presented parts of the results described in this thesis at the 64th European Atherosclerosis Society Congress (1994), Utrecht, the Netherlands, the 12th International Symposium on Drugs Affecting Lipid Metabolism (1995), Houston, Texas, the 68th Scientific Sessions of the American Heart Association (1995), Anaheim, California, and at the 66th European Atherosclerosis Society Congress (1996), Florence, Italy. He obtained the Young Investigator's Award during the October Meeting of the Nutrition Working Group of the Dutch Organization for Scientific Research (NWO) in 1995. He was selected as a participant in the third European Nutrition Leadership Program (1996) in Luxembourg, and was invited as a junior staff member for the fourth Program (1997).

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Appendix - Overview of coffee brewing methods

Coffee type	*Preparation technique*	*Brewing strength*	*Cup size*	*Remarks*
FILTERED	Boiled water is poured over finely ground, roasted coffee beans in a paper filter, either by hand or by using an electric coffee maker	25-75 g/L	110-190 mL	The filter is usually a paper filter, but re-usable filters can consist of nylon, cotton, or can be aluminum or gold-plated
PERCOLATED	Coarsely ground, roasted coffee beans are extracted by recirculating boiling water until the desired brew strength is reached	40-60 g/L	150-190 mL	The major type of coffee in the USA until about two decades ago
INSTANT	About 2 g of soluble coffee granules are dissolved into 150-190 mL of hot water		150-190 mL	Popular worldwide, but mostly so in Canada, Australia, and the UK
MOCHA	Just overheated water is forced through a bed of finely ground, usually dark roasted coffee beans	60 g/L	60 mL	Common in Italian and Spanish households
ESPRESSO	Hot water is forced under high pressure through a bed of finely ground, usually dark roasted coffee beans	up to 300 g/L	Italy:25-50 mL Other:25-150 mL	Common in Italy and Switzerland, but of growing popularity world-wide
BOILED	Coarsely ground, roasted coffee beans are boiled with water for 10 or more min, or infused with hot water, and the liquid is decanted without the use of a filter	ca 70 g/L	150 mL	Common in Poland. Common in Scandinavia until recently; in 1990, about 25% of Scandinavians still used boiled coffee
CAFETIERE	Hot water is poured onto coarsely ground, roasted coffee beans, and after 2 to 5 min of infusion the screen strainer is pushed down to separate the grounds from the fluid	up to 65 g/L	150-190 mL	Fairly uncommon, though its popularity is increasing. Known in the USA as **FRENCH PRESS** coffee
TURKISH	Very finely ground, roasted coffee beans are brought to a boil once or repeatedly, or infused with hot water (**MUD** coffee), and the liquid is decanted without the use of a filter	ca 80 g/L	60 mL	Also called **GREEK** coffee. Common in Eastern-European countries, Turkey, Greece, and the Middle-East including Israel.