



Diterpenes from coffee beans decrease serum levels of lipoprotein(a) in humans: results from four randomised controlled trials

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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 toward dietary interventions. We now experimentally studied the influence of coffee diterpenes on lipoprotein(a) levels.

Design: Four randomised controlled trials.

Subjects: Healthy, normolipidemic volunteers.

Interventions: Coffee, coffee oil, and pure diterpenes for 4–24 weeks.

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

Results: In 22 subjects drinking five to six strong cups of cafetiere 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/d of cafestol (plus kahweol)—the amount present in two to three cups of cafetiere 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.

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Descriptors: cafestol; experiment; human; kahweol; lipid metabolism; liver

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) (Dahlén, 1994). An extensive body of laboratory evidence indicates a critical role of Lp(a) in the development of atherothrombotic diseases (Marcovina & Morrisett, 1995), which is confirmed by most (Rosengren *et al*, 1990; Sigurdsson *et al*, 1992; Wald *et al*, 1994; Cremer *et al*, 1994; Bostom *et al*, 1994; Schaefer *et al*, 1994; Terres *et al*, 1995; Bostom *et al*, 1996), though not all (Jauhiainen *et al*, 1991; Ridker *et al*, 1993; Alftan *et al*, 1994; Ridker *et al*, 1995), prospective cohort studies. The circulating level of Lp(a) is largely under genetic control (Boerwinkle *et al*, 1992), and is unaffected by many interventions known to affect LDL metabolism, including most dietary interventions (Berglund, 1995).

The diterpenes cafestol and kahweol are lipids that are unique to coffee beans (Viani, 1986). They are responsible for the cholesterol-raising effect of Scandinavian boiled

coffee (Weusten-van der Wouw *et al*, 1994; Heckers *et al*, 1994). Cafestol and kahweol strongly affect lipid metabolism with short-term intake (Bak & Grobbee, 1989; Aro *et al*, 1987; van Dusseldorp *et al*, 1991; Ahola *et al*, 1991; Aro *et al*, 1990; Urgert *et al*, 1996a) as well as in life-long consumers of unfiltered coffee (Bönaa *et al*, 1988; Stensvold *et al*, 1989; Weusten-van der Wouw *et al*, 1994; Pietinen *et al*, 1990; Lindahl *et al*, 1991). 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 (Urgert *et al*, 1996b).

We now report the effects of preparations rich in cafestol and kahweol on Lp(a) levels in healthy, normolipidemic volunteers in four independent experiments. The effects on serum lipids and liver enzymes have been reported elsewhere (Weusten-van der Wouw *et al*, 1994; Urgert *et al*, 1996a; Urgert *et al*, 1997a).

Methods

Subjects

Participants were recruited through advertisements in newspapers and university buildings. Most subjects were young, nonobese, and normolipidemic (Table 1), and all

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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	21/15	10/0
Women using contraceptive steroid (<i>n</i>)	11	10	12	—
Smokers (<i>n</i>)	16	5	3	2
Age (y)	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

^aValues are numbers, or means ± s.d.

^bBody weights were measured without shoes or heavy clothing.

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 lifestyle, 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 (Urgert *et al*, 1995). 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 vs filtered coffee. This was a randomised, parallel controlled study. After a run-in period of four weeks on filtered coffee, subjects consumed 0.9 l of either filtered or cafetiere (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 (van Dusseldorp *et al*, 1991). Cafetiere 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.

Trials B and C—coffee oil vs placebo oil. These were double-blind, randomised parallel controlled studies. All participants first received for 1–2 weeks placebo oil which was a 3:2 (w/w) mixture of sunflower oil and palm oil. After randomization, 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 vs 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 seven 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 amino transferase. 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.

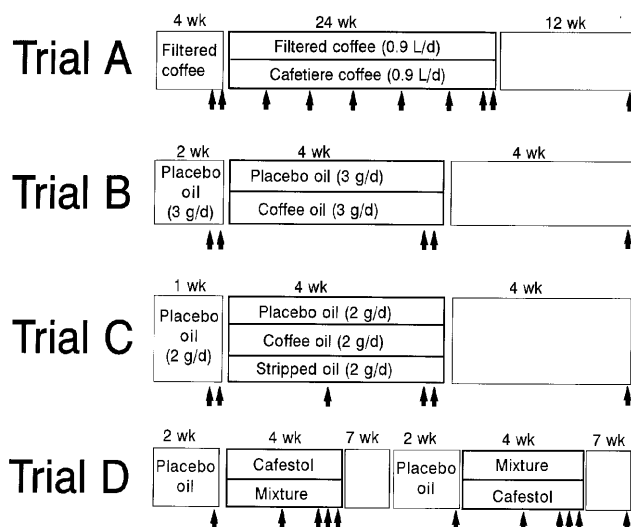


Figure 1 Experimental designs of four randomised studies with healthy normolipidemic volunteers. Arrows indicate times of blood sampling. Empty cells indicate wash-out and follow-up periods, during which no treatment was given.

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 1.7–84.0 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 immuno-sorbent assay (TintElize Lo(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 (Weusten-van der Wouw *et al*, 1994), and cafestol and kahweol in coffee preparations (Urgert *et al*, 1995), 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 vs filtered coffee. Daily consumption of five cups of cafetiere coffee produced a fall in Lp(a)

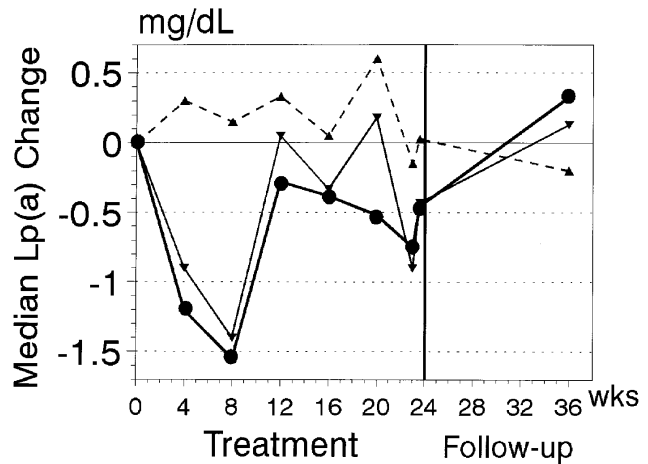


Figure 2 Trial A: Median changes in serum levels of lipoprotein(a) [Lp(a)] in subjects consuming five cups of cafetiere (▼, $n=22$) or filtered coffee (▲, $n=24$) per day for 24 weeks, and the difference between the changes (●) No treatment was given in the follow-up period.

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 vs 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.

Trial D—cafestol plus kahweol vs cafestol alone. The mixture of cafestol and kahweol as well as cafestol alone both reduced Lp(a) levels by 3–4 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 (s.e.m. 0.6) mg/dL (both $P>0.05$).

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

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 mg/day	Kahweol mg/day	Median mg/dL	Kahweol mg/dL	Median mg/dL	Mean \pm s.d. mg/dL
Trial A	Filtered coffee	24	1	9.2	20.8 \pm 22.3	0.3	0.2 \pm 0.8
	Cafetiere coffee	22	38	9.8	15.2 \pm 19.9	-0.9	-2.0 \pm 0.9*
Trial B	Placebo oil	16	0	17.2	25.9 \pm 23.8	0.5	1.1 \pm 0.9
	Coffee oil	16	85	14.9	29.1 \pm 32.7	-4.8**	-5.5 \pm 1.4**
Trial C	Placebo oil	15	0	17.7	24.4 \pm 23.4	0.8	-1.0 \pm 1.6
	Coffee oil	15	57	9.2	16.6 \pm 16.6	-2.3*	-4.5 \pm 1.3
	Coffee oil stripped of cafestol and kahweol	16	0	12.8	22.1 \pm 25.5	-0.3	-1.1 \pm 1.3
Trial D	Cafestol	10	63	11.5	13.9 \pm 7.5	-3.5 ^c	-3.5 \pm 0.8 ^c
	Cafestol plus kahweol	10	60	11.5	13.9 \pm 7.5	-3.1 ^c	-3.9 \pm 1.0 ^c

^aChanges were compared with Wilcoxon Rank Sum test and Mann Whitney U-tests for medians, and the *t*-tests for means.

^bResponse different from control group; * $P<0.05$; ** $P<0.01$.

^cResponse different from zero: $P<0.01$.

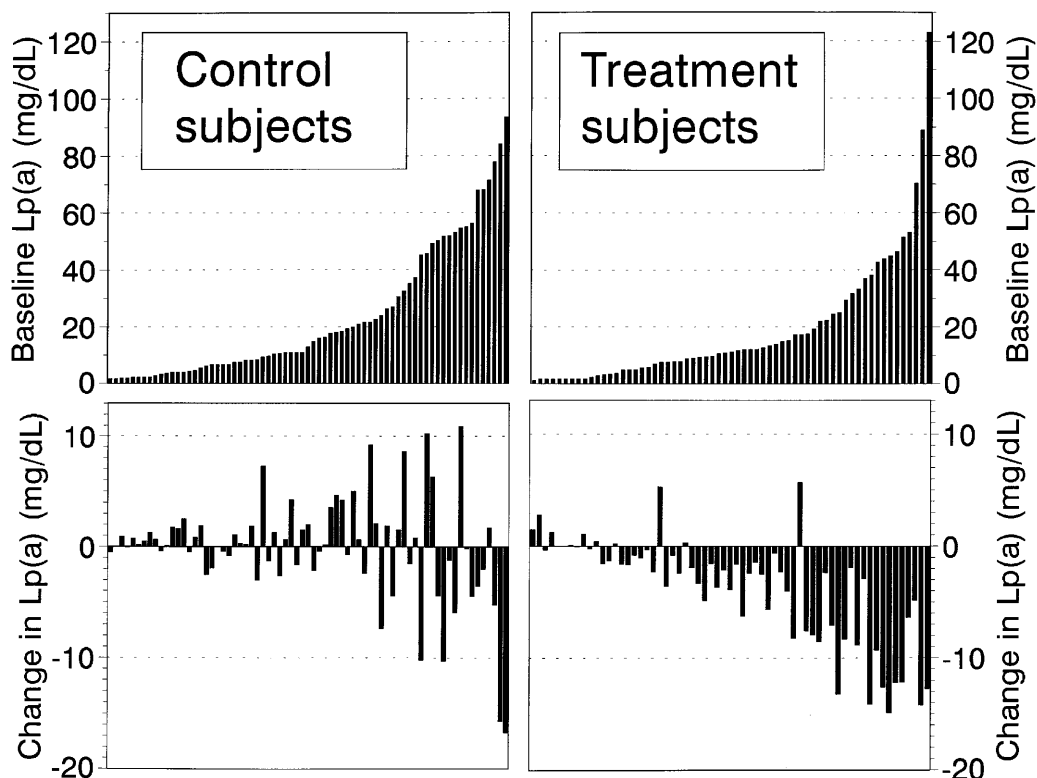


Figure 3 Individual baseline values (upper panels) and changes in serum lipoprotein(a) [Lp(a)] after four weeks (lower panels) in subjects who received placebo (control subjects, $n = 71$) or preparations rich in cafestol and kahweol (treatment subjects, $n = 63$) in four randomised, controlled trials.

(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.

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 one of the subjects took medications on a regular basis. Bias due to hormone intake or drug therapy (Berglund, 1995) 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 (Weustenvan der Wouw *et al*, 1994; Urgert *et al*, 1996a; Urgert *et al*, 1997a). 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 (Mensink *et al*, 1992; Nestel *et al*, 1992; Almendingen *et al*, 1995). Fish oils (Beil *et al*, 1991; Haglund *et al*, 1994) as well as high doses of ascorbic acid (Rath, 1992) were found to reduce Lp(a) levels, but other attempts could not verify this (Malle *et al*, 1991; Berg Schmidt *et al*, 1991; Salvi *et al*, 1993; Eritsland *et al*, 1995; Bostom *et al*, 1995). Coffee diterpenes are thus among the

few dietary components that modulate the circulating level of Lp(a).

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. Under the assumption that the relationship is

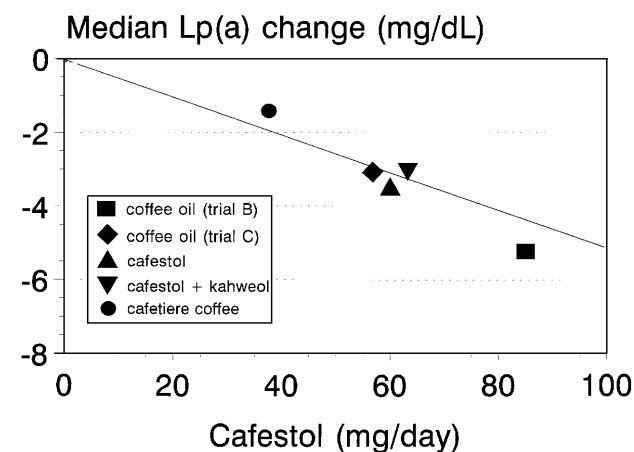


Figure 4 Relation of the observed median change in lipoprotein(a) [Lp(a)] after four weeks of treatment with the average cafestol intake per day (Table 2) across the four trials. The responses are given relative to the median changes in the respective control groups.

linear, each 10 mg—the amount present in two to three cups of cafetiere 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 cafetiere coffee. For espresso coffee, about six cups per day are needed for a similar effect (Urgert *et al*, 1995).

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 (Urgert & Katan, 1997b). The hypercholesterolemic 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 (Tverdal *et al*, 1990), 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 vs 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 (Urgert *et al*, 1996b).

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 (Berglund, 1995) 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 northern Norway, where boiled coffee is more common (Stensvold *et al*, 1989).

In trial A, Lp(a) levels were depressed by up to 15% in the first two months of intake of cafetiere 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 (Mensink *et al*, 1992; Nestel *et al*, 1992; Almendingen *et al*, 1995). 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 raises serum levels of alanine amino transferase (Weusten-van der Wouw *et al*, 1994; Urgert *et al*, 1995; Van Rooij *et al*, 1995; Urgert *et al*, 1996a), which may point at disturbed hepatocyte integrity (Keil, 1990). 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 (Kraft *et al*, 1989), and circulating levels of Lp(a) are largely determined by the rate of production (Rader *et al*, 1993). Indeed patients with biliary cirrhosis (Feely *et al*, 1992; Gregory *et al*, 1994; van Wesch, 1994) or other liver diseases (Gregory *et al*, 1994) have higher levels of lipids and liver amino-transferases, 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 (Weusten-van der Wouw *et al*, 1994) or espresso coffee (Casiglia *et al*, 1993) had no elevated levels of alanine amino transferase. 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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