

EFFECT OF DECAFFEINATED VERSUS REGULAR COFFEE ON SERUM LIPOPROTEINS

A 12-WEEK DOUBLE-BLIND TRIAL

MARIJKE VAN DUSSELDORP,¹ MARTIJN B. KATAN,^{1,2} AND
PIERRE N. M. DEMACKER³

van Dusseldorp, M. (Human Nutrition Section, Dept. of Medicine, U. of Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands), M. B. Katan, and P. N. M. Demacker. Effect of decaffeinated versus regular coffee on serum lipoproteins: a 12-week double-blind trial. *Am J Epidemiol* 1990;132:33-40.

Reports on the association between caffeine intake and serum cholesterol are inconsistent. In 1988, the authors examined the effect of decaffeinated versus regular coffee on serum lipids in 45 healthy volunteers from the general population living in or near Nijmegen, The Netherlands. Twenty-three women and 22 men aged 25-45 years with a habitual intake of 4-6 cups of regular coffee per day participated in a randomized double-blind crossover trial. They received five cups of regular coffee each day for a period of 6 weeks and five cups of decaffeinated coffee for the next 6 weeks, or vice versa. The background diet was kept constant and was low in caffeine. Differences between the effects of decaffeinated and regular coffee on blood lipids were essentially zero; the effect on serum total cholesterol (\pm standard deviation) was 0.01 (± 0.36) mmol/liter (0 \pm 14 mg/dl), that on high density lipoprotein cholesterol was 0.01 (± 0.11) mmol/liter (0 \pm 4 mg/dl), and that on triglycerides was 0.03 (± 0.29) mmol/liter (3 \pm 26 mg/dl). It was concluded that, in healthy adults, replacement of regular coffee by decaffeinated coffee has no effect on serum cholesterol and lipoproteins.

caffeine; cholesterol; clinical trials; coffee; lipoproteins; triglycerides

Coffee is the most widely used stimulant in Western society. In the Netherlands, 94 percent of adults drink at least one cup per day, and the per capita intake is 4.5 cups per day (1). An increasing proportion of consumers is switching from regular coffee to decaffeinated coffee. In the Netherlands,

the market share of decaffeinated coffee grew from 2 percent in 1984 to 4 percent in 1987 (1). In the United States, the proportion is already 20 percent (2). The switch is motivated partly by the well-documented negative effects of caffeine on the quality of sleep, and partly by other purported neg-

Received for publication May 30, 1989, and in final form October 19, 1989.

Abbreviation: SD, standard deviation.

¹ Human Nutrition Section, Department of Medicine, University of Nijmegen, Nijmegen, The Netherlands.

² Department of Human Nutrition, Agricultural University, Wageningen, The Netherlands.

³ Division of General Internal Medicine, University of Nijmegen, Nijmegen, The Netherlands.

Reprint requests to Marijke van Dusseldorp, Human Nutrition Section, Department of Medicine, Uni-

versity of Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands.

This study was financially supported by Netherlands Heart Foundation grant 87.026. The coffee was a gift of the Dutch Association of Coffee Roasters and Tea Packers.

The authors thank the dietitians, S. Meyboom, A. Severijnen-Nobels, and M. Willems, for conducting the interviews and the nursing and laboratory staff of the Department of Medicine for their assistance with blood sampling and analysis.

ative effects, including those on the cardiovascular system. However, surprisingly little is known about the actual effects of switching to decaffeinated coffee on risk factors for coronary heart disease.

Consumption of the "boiled" type of coffee traditionally consumed in Scandinavia is associated with elevations in serum cholesterol (3). Reports on the association between serum cholesterol concentrations and the intake of drip filter coffee, as commonly consumed in Western Europe and the United States, are much less consistent (4-8), and the possible role of caffeine in the association with serum cholesterol level remains unclear. Some epidemiologic studies reported a significant association between serum cholesterol and the intake of regular coffee but not of decaffeinated coffee (5, 6), or between serum cholesterol and caffeine content of the diet (8), while others found no significant relation between serum cholesterol and total caffeine intake (7). Epidemiologic studies on coffee and serum lipids often did not control for confounders like fat intake, or caffeine intake from other sources. Controlled studies have not, as yet, shown any evidence for a relation between caffeine consumption and serum cholesterol (9-12). However, these were small short-term studies with no dietary control. We have now compared the effect of regular and decaffeinated filter coffee on serum cholesterol in a 12-week randomized controlled trial with healthy volunteers. The effects of these treatments on blood pressure have been published elsewhere (13).

MATERIALS AND METHODS

Design

Our null hypothesis was that consumption of decaffeinated coffee instead of regular coffee for 5-6 weeks would not affect serum cholesterol. The alternative hypothesis was that decaffeinated coffee would lower serum cholesterol. We decided that the trial should have a statistical power of 85 percent to detect an effect on serum

cholesterol of 0.20 mmol/liter (8 mg/dl) at the $p < 0.05$ confidence level (two-tailed test). Calculations showed that we would need 46 subjects to meet these objectives.

The study comprised a randomized double-blind crossover trial with a 6-week study period on regular filter coffee followed by a 6-week period on decaffeinated filter coffee or vice versa. Subjects were randomized over the two treatment orders as follows: After admission, subjects were grouped by sex. Both groups were then divided into a subgroup of "high" serum cholesterol (median and above-median) and one of "low" serum cholesterol (below-median). Within each cell, subjects were grouped into pairs of similar age, and one member of each pair was randomly allocated to each treatment sequence.

Coffee cartons, each containing 10 packages of coffee (see below), were labeled by two persons not involved in the trial. The label carried the subject's name and number and the week of consumption. The project leader (M. v. D.), the research dietitians, and all other persons involved in the study, as well as the subjects, were blind to the kind of coffee consumed. In addition, subjects were blind to the study design; they did not know whether or how often they were switched between types of coffee.

During the trial, the subjects consumed two cups of either regular or decaffeinated coffee before noon, one cup in the afternoon, and two cups in the evening. Consumption of tea and other caffeine-containing products and drugs was prohibited, with the exception of chocolate, which was allowed in amounts containing up to 25 mg of caffeine per day. Once per week, the subjects visited a research dietitian, who checked food intake by a dietary recall, weighed the subject, gave out coffee cartons for the next week, and collected empty packages from the previous week. Subjects recorded in diaries any signs of illness, any medications used, amounts of chocolate eaten, and any deviation in coffee consumption. Twice-weekly contacts with the investigators, a weekly newsletter, and cov-

erage of the progress of the trial by the local media helped to keep up subjects' morale and motivation.

Subjects

The subjects were volunteers from the general population living in or near Nijmegen, a mixed industrial/college town of 150,000 inhabitants in the eastern Netherlands. They were recruited via publicity in local newspapers and through posters in university buildings. After having been thoroughly informed about the purpose and protocol of the study, 150 subjects declared themselves eager to participate and filled out a questionnaire. Out of these 150, 60 subjects (31 men and 29 women) met our criteria for initial eligibility—namely, age 17–45 years, apparently healthy, abstinence from smoking for the past year, no use of medication, not on a prescribed diet, no use of oral contraceptives, not pregnant, and a habitual consumption of 4–6 cups of regular coffee per day. These 60 subjects participated in a physical and laboratory examination and a 3-day dietary record. Fifteen proved ineligible; the reasons were serum cholesterol >6.7 mmol/liter (260 mg/dl) ($n = 9$), various medical reasons ($n = 3$), living too far from the clinic ($n = 2$), or job change ($n = 1$). The remaining 45 subjects (23 women and 22 men) were admitted to the study. Ten were employed by the university, five by other educational institutions, two by the municipality, and 12 by other employers. Another five were students, 10 were housewives, and one was

unemployed. Table 1 shows the subjects' baseline characteristics; 38 percent had a borderline high (5.2–6.2 mmol/liter or 200–240 mg/dl) and 18 percent a high (6.2 mmol/liter or >240 mg/dl) blood cholesterol level by present US standards.

The protocol for the study, which had been approved by the local ethical committee, was explained to the volunteers, and all subjects gave their written informed consent. Subjects were asked to maintain their usual pattern of activity and to keep up a stable body weight.

Coffee

The coffee used was similar to the most popular types of regular and decaffeinated coffee sold in the Netherlands, but it was packaged especially for the trial in blank packages. The coffee was supplied in single-cup disposable packages which contained (mean \pm standard deviation (SD)) 5.4 ± 0.1 g of coffee for the regular coffee and 5.1 ± 0.2 g for decaffeinated coffee, the difference being caused by the process of extraction of caffeine with dichloromethane. The regular coffee was composed of 71 percent arabica and 29 percent robusta beans, and the decaffeinated coffee was made of 58 percent arabica and 42 percent robusta beans. At that time, these were regular blends of the vendor who supplied the coffee. Each package fitted into the bottom of a plastic holder which could be placed on top of a cup or beaker. Hot water (110–150 ml) was poured into the holder, and it then dripped through the coffee package and its

TABLE 1

Baseline characteristics of 45 subjects in a study of caffeine's effect on serum cholesterol, Nijmegen, The Netherlands, November and December 1987*

Variable	Mean	SD†	Range
Age (years)	38	7	25–45
Body mass index (weight (kg)/height (m) ²)	23.2	4.0	18.8–31.3
Cholesterol (mmol/liter)			
Total	5.56	0.94	4.32–6.65
HDL†	1.52	0.60	0.85–2.77
Triglycerides (mmol/liter)	1.23	0.80	0.57–2.89

* Thirty days before the experiment.

† SD, standard deviation; HDL, high density lipoprotein.

filter paper bottom into the cup. The regular and decaffeinated coffee were similar in taste. We prepared 60 cups of coffee from random packs and analyzed them in duplicate for caffeine. The mean amount \pm SD was 83.5 ± 12.6 mg/cup for the regular coffee and 3.1 ± 0.3 mg/cup for the decaffeinated coffee. The subjects recorded each day in their diaries which kind of coffee they thought they were receiving.

Blood sampling and analysis

Fasting blood samples were obtained on days 16, 35, and 42 of each treatment period. An additional sample was collected on day 22 at midday to check for caffeine. Cholesterol and triglycerides in whole serum were determined with enzymatic methods on a Hitachi 717 analyzer using CHOD-PAP reagent no. 1040839 and GPO-PAP reagent no. 1058550, provided by Boehringer Mannheim (Mannheim, Federal Republic of Germany). High density lipoprotein cholesterol was determined after precipitation of very low density lipoprotein and low density lipoprotein from whole serum with polyethylene glycol 6,000 (14). Low density lipoprotein cholesterol was calculated (15). The within-day coefficient of variation was 1 percent for total and high density lipoprotein cholesterol and 2 percent for triglycerides. Control pools of known value from the Centers for Disease Control (Atlanta, GA) yielded values within 5 percent of the Centers for Disease Control target values.

Serum caffeine was measured by reversed-phase high-performance liquid chromatography (16).

Statistical analysis

The lipid values and serum caffeine levels on regular and on decaffeinated coffee, as well as the differences in these variables between the two treatment periods, were all normally distributed (17). An exact test based on a *t* distribution and on pooled estimates of variance (18) indicated that carryover and period effects were absent.

Treatment effects were calculated for each subject as the change from the means of weeks 5 and 6 of the decaffeinated coffee period to those of the regular coffee period; treatment effects were examined by a two-sided *t* test (18).

RESULTS

Compliance and blinding

All 45 subjects completed the experiment successfully. Both the diaries and frequent personal interviews indicated excellent adherence to the protocol. Empty packages were returned by the subjects for 99.7 percent of all 18,900 coffee packages distributed. The mean \pm SD of the caffeine concentration in serum collected on the 22nd day of each period between noon and 2:00 p.m. was 3.2 ± 1.5 mg/liter (range, 0.3–7.0) when subjects consumed regular coffee and 0.2 ± 0.3 mg/liter (range, 0.0–1.2) when subjects consumed decaffeinated coffee. One subject had one value over 1.0 on decaffeinated coffee, and one other subject had one value below 1.0 on caffeinated coffee. Both subjects showed levels in the expected range on the seven other blood sampling occasions; we therefore ascribed the two outliers to chance. Subjects apparently remained unable to tell which type of coffee they received. According to the diaries, when subjects were receiving regular coffee, they correctly identified the type of coffee on 54 percent of the days, they were wrong on 19 percent of the days, and they could not tell on 27 percent of the days. When subjects received decaffeinated coffee, these percentages were 27, 46, and 27, respectively. Therefore, according to the subjects' diaries, they thought they were drinking regular coffee on 23 out of 42 days on which they received regular coffee and on 19 out of the 42 days on which they received decaffeinated coffee. Evidently, very few subjects were able to recognize the switch from caffeinated coffee to decaffeinated or vice versa. Total caffeine intake, including that from chocolate, was 435 mg/day on regular coffee and 25 mg/day on decaffeinated coffee.

fee. According to the dietary recalls, differences in nutrient intake between treatment periods were negligible (table 2). The mean change in body weight from weeks 5 and 6 to weeks 11 and 12 was 0.14 kg (range, -2.8 to 1.9 kg). The value of -2.8 was from one man who developed a fever due to bronchitis during weeks 11 and 12. For all other participants, the change in body weight was less than 2 kg.

Serum lipoprotein lipids

The changes in serum total cholesterol and high density lipoprotein cholesterol during the experiment are shown in figure 1. Mean changes in serum lipid values from one treatment period to another were essentially zero. Treatment effects on serum total cholesterol, high and low density lipoprotein cholesterol, and triglycerides had very narrow 95 percent confidence intervals centering around zero (table 3). Separate analyses based on comparing effects after

4, after 5, and after 6 weeks of treatment or after exclusion of the subject who became ill yielded the same results. The results were similar for men and women.

DISCUSSION

Our results indicate that chronic consumption of five cups of decaffeinated coffee per day in comparison with regular coffee has no effect on serum cholesterol in healthy men and women. Compliance was very high: 99.7 percent of all coffee packages distributed were returned by the subjects, and the mean serum caffeine concentration during the day was 16-fold higher when subjects were consuming regular coffee than when they were consuming decaffeinated coffee. The fact that subjects were unable to correctly identify the type of coffee they were consuming shows that blinding was effective. In addition, the absence of changes in body weight, nutrient intakes,

TABLE 2

Intakes (mean \pm standard deviation) of energy and nutrients in 45 subjects in a study of caffeine's effect on serum cholesterol, January-April 1988, Nijmegen, The Netherlands

Variable	Pre-experimental*	Decaffeinated†	Caffeinated‡	Δ (Decaffeinated - caffeinated)
Energy (kcal/day)‡	2,288 \pm 745	2,467 \pm 795	2,448 \pm 833	19 \pm 333
Protein (% of energy)	14 \pm 3	14 \pm 3	14 \pm 3	0 \pm 1
Fat, total (% of energy)	38 \pm 7	39 \pm 8	38 \pm 8	1 \pm 4
Saturated fatty acids (% of energy)	16 \pm 4	16 \pm 4	16 \pm 4	0 \pm 2
Monounsaturated fatty acids (% of energy)	14 \pm 4	15 \pm 4	15 \pm 4	0 \pm 2
Polyunsaturated fatty acids (% of energy)	6 \pm 3	8 \pm 3	7 \pm 3	1 \pm 2
Carbohydrate, total (% of energy)	46 \pm 8	45 \pm 8	45 \pm 9	0 \pm 4
Mono- and disaccharides (% of energy)	22 \pm 7	21 \pm 6	21 \pm 7	0 \pm 3
Alcohol (% of energy)	4 \pm 4	3 \pm 5	4 \pm 5	0 \pm 2§
Dietary fiber (g/day)	29 \pm 12	31 \pm 13	31 \pm 14	0 \pm 4
Cholesterol (g/day)	0.32 \pm 0.2	0.33 \pm 0.2	0.31 \pm 0.2	0.02 \pm 0.1
Calcium (g/day)	1.16 \pm 0.5	1.19 \pm 0.5	1.21 \pm 0.5	-0.02 \pm 0.2
Sodium (g/day)	2.68 \pm 1.2	2.99 \pm 1.3	3.02 \pm 1.3	-0.03 \pm 0.6
Potassium (g/day)	3.74 \pm 1.1	3.57 \pm 1.3	3.61 \pm 1.3	-0.05 \pm 0.4

* Pre-experimental food intake was assessed by a 3-day weighed record method.

† Intakes during the trial represent means of six 24-hour recalls during each study period.

‡ Mean daily energy intake was 2,857 kcal/day for men and 2,083 kcal/day for women.

§ Difference of 1 caused by rounding.

|| From food only; added salt not measured.

or amount of physical exercise, as indicated by the diaries, indicated good adherence to the protocol.

Coffee contains cholesterol-raising elements, as is shown by the significant associations between boiled coffee intake and serum cholesterol level found in several controlled trials (19–21) and in an observational study (3). However, no association was found for filter coffee in any of these studies. Caffeine intake was significantly associated with serum cholesterol levels in a cross-sectional study of 4,757 Australians (8). This association, however, was seen only in women, and a significant interaction between smoking and caffeine consumption was found in their association

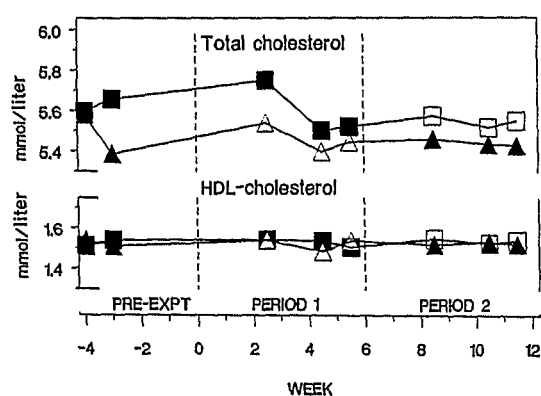


FIGURE 1. Effect of drinking five cups of decaffeinated or regular filter coffee per day for a period of 6 weeks each on serum total and high density lipoprotein (HDL) cholesterol in 45 healthy volunteers, January–April 1988, Nijmegen, The Netherlands. Each symbol represents the mean of 22 (11 men, 11 women) (Δ) or 23 (11 men, 12 women) (\square) subjects. Closed symbols represent regular coffee use and open symbols decaffeinated coffee use.

with serum cholesterol levels. These and results of other epidemiologic studies suggest synergism between the effects of coffee or caffeine consumption and cigarette smoking on serum lipids (22, 23). Since smokers were excluded from participation in the present trial, our conclusions might apply only to nonsmokers.

In other observational studies by Haffner et al. (5) and Mathias et al. (6), plasma cholesterol levels were significantly associated with the intake of regular coffee but not of decaffeinated coffee. However, in these studies, the numbers of subjects consuming decaffeinated coffee only were too small to investigate the effect of caffeine on serum cholesterol. In the study by Curb et al. (24), serum cholesterol level was significantly associated with caffeine intake and coffee consumption, but not with tea or cola consumption. The authors suggested that the caffeine-cholesterol relation is primarily due to the contribution of coffee consumption to caffeine intake. It remains possible, however, that tea and cola contain other substances that balance the hypercholesterolemic effect of caffeine. Results of a study by Davis et al. (7) also showed a significant association between coffee consumption and serum cholesterol, while that between total caffeine intake and serum cholesterol failed to reach the $p = 0.05$ level of significance. The authors suggested that there may be something in coffee besides caffeine that is related to elevations in serum cholesterol level. However, findings were not adjusted for dietary

TABLE 3

Effects (mean \pm standard deviation) of drinking five cups of decaffeinated or regular coffee for periods of 6 weeks each on plasma lipids and lipoproteins ($n = 45$), January–April 1988, Nijmegen, The Netherlands

Type of coffee	Total cholesterol (mmol/liter)	HDL* cholesterol (mmol/liter)	LDL* cholesterol (mmol/liter)	Total triglycerides (mmol/liter)
Decaffeinated	5.48 \pm 0.79	1.52 \pm 0.40	3.40 \pm 0.80	1.20 \pm 0.52
Regular	5.47 \pm 0.74	1.52 \pm 0.40	3.41 \pm 0.74	1.17 \pm 0.53
Difference	0.01 \pm 0.36 (-0.10 to 0.12) \dagger	0.01 \pm 0.11 (-0.02 to 0.04)	-0.01 \pm 0.31 (-0.10 to 0.08)	0.03 \pm 0.29 (-0.06 to 0.12)

* HDL, high density lipoprotein; LDL, low density lipoprotein.

\dagger Numbers in parentheses, 95 percent confidence interval.

intake of fatty acids and cholesterol. In addition, the study may have failed to pick up an actual association between total caffeine intake and serum cholesterol, because the observed significance level was only slightly above the 0.05 level (0.08).

Since it is not possible to infer a causal relation from cross-sectional studies, controlled trials are needed. So far, three controlled trials have shown no significant effect of caffeine on serum cholesterol level (9, 10, 12); one even suggested a negative relation between caffeine and serum cholesterol (11). These studies, however, have not been conclusive on this point, since treatment periods in these studies were short (from 2 hours to 20 days) or the numbers of subjects were small, yielding a low power with the chance of missing clinically important differences. Dietary control has also been lacking.

One trial showed a significant increase in low density lipoprotein cholesterol and apolipoprotein B 2 months after subjects had switched from drinking regular coffee to drinking decaffeinated coffee (25). The method of brewing was not reported. The senior author suggested that the effect on low density lipoprotein cholesterol was caused not by the absence of caffeine but by the fact that robusta beans are used for making decaffeinated coffee and arabica beans for regular coffee (press release, Stanford University Medical Center, November 9, 1989). Our results do not support this suggestion, because decaffeinated coffee did not elevate low density lipoprotein cholesterol levels even though it contained a higher proportion of robusta beans than the regular coffee.

Our study shows that consumption of five cups of decaffeinated coffee per day instead of regular coffee does not affect serum total cholesterol, high density lipoprotein cholesterol, or serum triglycerides in healthy, nonsmoking men and women with normal or mildly elevated serum cholesterol over a 6-week period. Since the power of this study to detect a difference of 0.20 mmol/liter (8 mg/dl) was 85 percent

and the 95 percent confidence intervals are narrow, there is no reason to suspect the trial of being too small to detect clinically important differences. Therefore, it seems unnecessary for hypercholesterolemic patients to switch from regular to decaffeinated coffee out of fear that caffeine elevates low density lipoprotein cholesterol; the present study shows that such a switch has no effect on serum lipid levels. Our study does not preclude the possibility that total abstinence from regular filter coffee will affect serum lipids. It does strongly suggest that caffeine is not the component of boiled coffee responsible for its cholesterol-elevating effect. The question thus remains as to what the substances are in boiled coffee that affect cholesterol metabolism.

REFERENCES

1. Vereniging van Nederlandse Koffiebranders en Theepakkers. Jaarverslag, 1987. Amsterdam: Vereniging van Nederlandse Koffiebranders en Theepakkers, 1988:28. (Association of Dutch Coffee Roasters and Tea Packers. Annual report, 1987. Amsterdam: Association of Dutch Coffee Roasters and Tea Packers, 1988:28.)
2. International Coffee Organization. United States of America, coffee drinking study—winter 1982. London: International Coffee Organization, 1982.
3. Bønaa K, Arnesen E, Thelle DG, et al. Coffee and cholesterol: Is it all in the brewing? The Tromsø Study. *Br Med J* 1988;297:1103-4.
4. Thelle DS, Heyden S, Fodor JG. Coffee and cholesterol in epidemiological and experimental studies. *Atherosclerosis* 1987;67:97-103.
5. Haffner SM, Knapp JA, Stern MP, et al. Coffee consumption, diet, and lipids. *Am J Epidemiol* 1985;122:1-12.
6. Mathias S, Garland C, Barrett-Connor E, et al. Coffee, plasma cholesterol, and lipoproteins: a population study in an adult community. *Am J Epidemiol* 1985;121:896-905.
7. Davis BR, Curb D, Borhani NO, et al. Coffee consumption and serum cholesterol in the Hypertension Detection and Follow-up Program. *Am J Epidemiol* 1988;128:124-36.
8. Shirlow MJ, Mathers CD. Caffeine consumption and serum cholesterol levels. *Int J Epidemiol* 1984;13:422-7.
9. Bellet S, Kershblum A, Aspe J. The effect of caffeine on free fatty acids. *Arch Intern Med* 1965;116:750-2.
10. Aro A, Kostiaimen E, Huttunen JD, et al. Effects of coffee and tea on lipoproteins and prostanoids. *Atherosclerosis* 1985;57:123-8.
11. Naismith DJ, Akinyanju PA, Szanto S, et al. The effect in volunteers of coffee and decaffeinated coffee on blood glucose, insulin, plasma lipids, and some factors involved in blood clotting. *Nutr*

- Metab 1970;12:144-51.
12. Paoletti R, Corsini A, Tremoli E, et al. Effects of coffee on plasma lipids, lipoproteins, and apolipoproteins. *Pharmacol Res* 1989;21:27-38.
 13. van Dusseldorp M, Smits P, Thien T, et al. Effect of decaffeinated versus regular coffee on blood pressure: a 12-week, double-blind trial. *Hypertension* 1989;14:563-9.
 14. Demacker PNM, Hijmans AGM, Vos-Janssen HE, et al. A study of the use of polyethylene glycol in estimating cholesterol in high-density lipoproteins. *Clin Chem* 1980;26:1775-9.
 15. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;18:499-502.
 16. Smits P, Thien T, van 't Laar A. Circulatory effects of coffee in relation to the pharmacokinetics of caffeine. *Am J Cardiol* 1985;56:958-63.
 17. SAS Institute, Inc. SAS user's guide: basics. 5th ed. Cary, NC: SAS Institute, Inc, 1985:1187.
 18. Hills M, Armitage P. The two-period cross-over clinical trial. *Br J Clin Pharmacol* 1979;8:7-20.
 19. Arnesen E, Førde OH, Thelle DS. Coffee and serum cholesterol. *Br Med J* 1984;288:1960.
 20. Førde OH, Knutsen SF, Arnesen E, et al. The Tromsø Heart Study. Coffee consumption and serum lipid concentrations in men with hypercholesterolaemia: a randomised intervention study. *Br Med J* 1985;290:893-5.
 21. Aro A, Tuomilehto J, Kostianen E, et al. Boiled coffee increases serum low density lipoprotein concentration. *Metabolism* 1987;36:1027-30.
 22. Heyden S, Heiss G, Manegold C, et al. The combined effect of smoking and coffee drinking on LDL and HDL cholesterol. *Circulation* 1979;60:22-5.
 23. Salonen JT, Happonen P, Salonen R, et al. Interdependence of associations of physical activity, smoking, and alcohol and coffee consumption with serum high-density lipoprotein and non-high-density lipoprotein cholesterol: a population study in eastern Finland. *Prev Med* 1987;16:647-58.
 24. Curb JD, Reed DM, Kautz JA, et al. Coffee, caffeine, and serum cholesterol in Japanese men in Hawaii. *Am J Epidemiol* 1986;123:648-55.
 25. Superko HR, Bortz WM, Albers JJ, et al. Lipoprotein and apolipoprotein changes during a controlled trial of caffeinated and decaffeinated coffee drinking in men. (Abstract). *Circulation* 1989;80:II86.