

## Cholesterol-Raising Factor From Boiled Coffee Does Not Pass a Paper Filter

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Previous studies have indicated that consumption of boiled coffee raises total and low density lipoprotein (LDL) cholesterol, whereas drip-filtered coffee does not. We have tested the effect on serum lipids of consumed coffee that was first boiled and then filtered through commercial paper coffee filters. Sixty-four healthy volunteers consumed six cups per day of this boiled and filtered coffee for 17 days. Then, they were randomly divided into three groups, which, for the next 79 days, received either unfiltered boiled coffee (lipid content, 1.0 g/l), boiled and filtered coffee (0.02 g lipid/l), or no coffee. Serum total cholesterol levels rose by 0.42 mmol/l (16 mg/dl; 95% confidence interval [CI], 0.14–0.71), LDL cholesterol levels by 0.41 mmol/l (16 mg/dl; 95% CI, 0.16–0.66), and apolipoprotein B levels by 8.6 mg/dl (95% CI, 3.8–13.4) in those who consumed boiled coffee relative to those who consumed boiled and filtered coffee. Responses of triglycerides, high density lipoprotein cholesterol, and apolipoprotein A-I did not differ significantly among these groups. No significant effects on serum lipid levels were found in the boiled and filtered coffee-consuming group compared with those who drank no coffee. In subjects who drank boiled coffee, serum campesterol level, an indicator of cholesterol absorption, remained constant. The serum lathosterol level, an indicator of cholesterol synthesis, increased by 11% ( $p < 0.05$ ), but the lathosterol to cholesterol ratio did not change. We propose that paper filters of the type used for drip-filtered coffee retain the lipid present in boiled coffee and in that way remove the hypercholesterolemic factor. Increased synthesis or absorption of cholesterol could not be shown to be the underlying mechanism by which consumption of boiled coffee raises cholesterol levels. (*Arteriosclerosis and Thrombosis* 1991;11:586–593)

The consumption of Scandinavian-style "boiled" coffee, made by boiling ground coffee with water and decanting the fluid into a cup, is strongly associated with the level of serum cholesterol,<sup>1,2</sup> and controlled experiments have confirmed a cause-and-effect relation.<sup>3–6</sup> The mechanism by which boiled coffee affects cholesterol metabolism is unclear. Caffeine, at least, is not the substance that raises cholesterol levels.<sup>7–9</sup> In a recent Dutch experiment,<sup>6</sup> cholesterol-raising boiled-type coffee was prepared by pouring water that had been brought to a boil onto ground coffee beans in a Thermos flask and letting the mixture stand for about 10 minutes. Thus, ground

coffee evidently does not need to be actively boiled in water to produce a hypercholesterolemic brew.

We<sup>10</sup> recently isolated a lipid-rich fraction from boiled coffee that markedly raised cholesterol levels in volunteers, and we suggested that filtered coffee does not raise serum cholesterol levels because it has a low lipid content. We now present a direct test of this hypothesis. We prepared cholesterol-raising, boiled-type coffee and tested both its lipid content and its effect on serum cholesterol level before and after filtration through a paper filter in a placebo-controlled trial involving 64 healthy subjects. We also measured serum levels of lathosterol and plant sterols, indicators of cholesterol synthesis and absorption, respectively, as a first approach to the mechanism of action of boiled coffee. The effects of the various treatments on blood pressure and heart rate will be described elsewhere (M. van Dusseldorp et al, unpublished observations).

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### Methods

#### Design

The study lasted 14 weeks, from September 11 to December 15, 1989. During a run-in period of 17 days, all subjects consumed six cups (0.9 l; 30.4 fl.

oz.) of boiled and filtered coffee per day. During the next 79 days, they consumed either 0.9 l of boiled coffee, 0.9 l of boiled and filtered coffee, or no coffee at all. Before randomization, subjects were grouped according to sex and serum cholesterol level (above/below median), which yielded four cells. Within each cell, the subjects were then grouped into triplets of similar age, and each member of a triplet was randomly allocated to a different treatment.

All subjects received two cups of herbal tea (Kneipp, Würzburg, F.R.G., and Salus Haus, Bruckmühl, F.R.G.) during the run-in period as an additional caffeine-free source of fluid. Subjects in the boiled and boiled and filtered coffee-consuming groups continued their consumption of two cups of herbal tea per day during the entire study period. During the test period, subjects in the no-coffee group received one glass of orange or apple juice, one glass of mineral water, and four cups of herbal tea per day. Only nonmedicinal herbal teas that complied with the strict safety regulations of the Federal Republic of Germany were used. The teas mainly consisted of mint, rosehips, chamomile, apple, blackberry, and hibiscus. The coffee, paper filters, coffee milk, herbal teas, juices, and mineral water were provided by us. In the no-coffee group, the effect of abstaining from coffee on serum cholesterol levels might have been confounded by coffee milk being eliminated from the diet simultaneously with the coffee. Therefore, those subjects randomized into the no-coffee group who used milk in their coffee were supplied with special cookies. During the run-in period, we provided these subjects with partly skimmed coffee milk, rich in polyunsaturated fatty acids, and with one special cookie per day. During the test period, they consumed a similar cookie that was enriched with polyunsaturated fatty acids to compensate for the polyunsaturated fatty acids in the 40 ml of coffee milk that they had used daily during the run-in period.

For all subjects, consumption of tea and other caffeine-containing products and preparations was prohibited, with the exception of chocolate, which was allowed in amounts providing as much as 15 mg caffeine per day, equivalent to 15 g of chocolate. Once a week, subjects visited a dietitian who checked food intake by a 24-hour dietary recall; weighed the subject; gave out his or her packs of coffee, paper filters, herbal tea, mineral water, juice, and cookies for the next week; and collected the empty packages from the previous week. Subjects recorded in diaries any signs of illness, medications used, amount of chocolate eaten, and any deviation from treatment. Frequent contacts with the investigators and a weekly newsletter helped to sustain the volunteers' morale.

#### Statistical Analysis

Prior calculations showed that, to detect a 0.26-mmol/l effect of drinking boiled or boiled and filtered coffee on serum cholesterol (with a power of 80% [ $p < 0.05$ ]), we would need 66 subjects. The response to the various treatments was calculated for each

subject as the change in serum lipid levels from the end of the run-in period (mean of days 12 and 17) to the end of the test period (mean of days 89, 93, and 96). Differences among groups and differences among groups adjusted for changes in body weight during the test period were analyzed by multiple regression.<sup>11</sup> When analysis indicated a significant treatment effect ( $p < 0.05$ ), the responses to boiled or no coffee were compared with the response of the group drinking boiled and filtered coffee. As this involved two simultaneous comparisons, probability was set at 0.025 instead of 0.05.  $p$  values are two tailed. Within each group, responses of men and women were compared by two-sided  $t$  test. Responses in serum cholesterol were adjusted for changes in intake of fatty acids and cholesterol by the formula of Keys et al.<sup>12</sup>

The changes in serum lathosterol and plant sterols in the boiled coffee-consuming group were not normally distributed; treatment effects were therefore examined by Wilcoxon's signed-rank test.

#### 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 section of The Netherlands. They were recruited via publicity in local newspapers and through posters in university buildings. After they were thoroughly informed about the purpose and protocol of the study, 167 subjects declared themselves eager to participate and filled out a questionnaire. Fifty men and 50 women met our criteria for initial eligibility, namely, 17–57 years old, apparently healthy, abstinent from smoking for the past year, not on medication, not on a prescribed diet, not using oral contraceptives, not pregnant, and with a habitual consumption of four to seven cups of regular drip-filtered coffee per day. These 100 subjects proceeded to a physical and laboratory examination, and they recorded their fluid intake for 5 days. Nineteen subjects proved ineligible because of high blood lipid levels ( $n=4$ ), blood pressure ( $n=6$ ), or body mass index ( $n=3$ ), or because of various medical ( $n=4$ ) or nonmedical ( $n=2$ ) reasons. Furthermore, 15 subjects were not admitted to the study because they drank fewer than three or more than seven cups of coffee per day or consumed more than five alcoholic drinks per day. The remaining 33 women and 33 men were admitted to the study. All subjects had completed secondary school, and 52% had completed college. Two women withdrew from the study during the run-in period, one for medical reasons and one for personal reasons. Thus, data from 64 subjects were analyzed. Table 1 provides their baseline characteristics.

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

TABLE 1. Baseline Characteristics of Subjects According to Treatment Group

| Characteristic                       | Treatment group      |                                   |                  |
|--------------------------------------|----------------------|-----------------------------------|------------------|
|                                      | Boiled coffee (n=22) | Boiled and filtered coffee (n=21) | No coffee (n=21) |
| Sex (M/F)                            | 11/11                | 11/10                             | 11/10            |
| Age (yr)                             | 39±6                 | 39±9                              | 39±8             |
| Body mass index (kg/m <sup>2</sup> ) | 23±2                 | 24±3                              | 22±2             |
| Serum cholesterol (mmol/l)           |                      |                                   |                  |
| Total                                | 5.4±0.6              | 5.3±0.8                           | 5.3±0.8          |
| HDL                                  | 1.6±0.4              | 1.4±0.3                           | 1.5±0.3          |
| LDL                                  | 3.4±0.7              | 3.4±0.8                           | 3.3±0.8          |
| Serum triglycerides (mmol/l)         | 1.0±0.3              | 1.2±0.4                           | 1.1±0.3          |

All values (except those for sex) are mean±SD. To convert from millimoles per liter to milligrams per deciliter, multiply cholesterol values by 38.67 and triglyceride values by 88.54.

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

### Coffee Preparation

The subjects were instructed to prepare and drink 0.9 l or six cups of coffee brew a day. Boiled-type coffee was prepared by pouring 0.5 l of boiling water onto 25 g of coarsely ground coffee (Roodmerk, Douwe Egberts, Utrecht, The Netherlands) in a 0.5-l Thermos flask. Ten minutes later, the liquid was decanted into a second Thermos flask, from which the coffee beverage was poured out into a cup or beaker just before consumption. One batch provided three cups of 0.15 l (5 fl. oz.), prepared with 8 g of ground coffee per cup. The contents of one bottle were usually consumed within half a day, after which another bottle was prepared. Most of the coffee grounds stayed behind in the first and second Thermos bottles. Those grounds that made their way into the cup were discarded by the study subjects.

Boiled and filtered coffee was prepared like the boiled coffee, but instead the liquid was poured into the second Thermos flask through a white paper filter (Melitta Nederland, Veenendaal, The Netherlands, 1×4, No. 4006508 200016) held in a conical plastic filter holder. Subjects in both groups were allowed to dilute the brew in the second Thermos bottle with hot water if they found it too strong for their taste.

The caffeine content, measured by reversed-phase high-performance liquid chromatography,<sup>13</sup> was 860 mg/l for the boiled and 887 mg/l for the boiled and filtered coffee.

### Blood Sampling and Analysis

Fasting blood samples were obtained on days -100 and -95 (during selection of volunteers); on days 0, 12, and 17 (during the run-in period); on days 45, 68, 89, 93, and 96 (test period); and 40 days after the trial had ended. Nonfasting samples were collected in the afternoon on days 32, 58, and 82 to check caffeine levels. Serum was stored at -80°C. Cholesterol and triglyceride values were determined by use of a Hitachi Model 717 discrete analyzer (Boehringer Mannheim, Mannheim, F.R.G.) with CHOD-PAP reagent

TABLE 2. Mean Changes in Body Weight and in Intake of Energy and Different Fatty Acids and Dietary Cholesterol According to Treatment Group

| Variable                                  | Treatment group      |                                   |                  |
|---|----------------------|-----------------------------------|------------------|
|   | Boiled coffee (n=22) | Boiled and filtered coffee (n=21) | No coffee (n=21) |
| Body weight (kg)                          | 0.4±1.0              | 0.2±1.8                           | 0.6±1.7          |
| Energy (MJ)                               | 1.1±1.5              | -0.1±1.9                          | -0.1±1.8         |
| Energy (kcal)                             | 269±367              | -34±444                           | -24±425          |
| Total fat (% of energy)                   | 2.1±5.5              | 0.8±5.9                           | 1.5±6.9          |
| Saturated fatty acids (% of energy)       | 0.0±2.8              | 0.3±2.8                           | 0.4±2.8          |
| Monounsaturated fatty acids (% of energy) | 1.1±3.2              | 0.2±2.9                           | 0.2±3.0          |
| Polyunsaturated fatty acids (% of energy) | 0.6±2.4              | 0.0±2.1                           | 0.5±3.2          |
| Cholesterol (mg/MJ)                       | -4.2±11.1            | 2.6±9.5                           | -5.0±15.2        |

Values are mean±SD. Changes were recorded for the 11.5-week period from the end of the run-in to the end of the test period.

No. 1040839 and GPO-PAP reagent No. 1058550, respectively (Boehringer Mannheim). High density lipoprotein (HDL) cholesterol was determined after precipitation of very low density lipoprotein (VLDL) and low density lipoprotein (LDL) cholesterol with polyethylene glycol 6000.<sup>14</sup> LDL cholesterol levels were calculated.<sup>15</sup> All samples obtained from one subject during the study period (days 0-96) were analyzed within one run. The coefficient of variation of control serum samples within one run was 1.4-1.9% for cholesterol, 1.9-2.5% for HDL cholesterol, and 1.9-2.1% for triglycerides. Control pools from the Centers for Disease Control (Atlanta, Ga.) yielded values within 2% of Centers for Disease Control target values. Apolipoproteins A-I and B were assayed by turbidimetry on microtiter plates.<sup>16</sup> All samples from one proband were measured on the same day within one series. The coefficient of variation was 3.5% for apolipoprotein A-I and 3.6% for apolipoprotein B within series and 3.1% for apolipoprotein A-I and 5.3% for apolipoprotein B between days.

Lathosterol, a cholesterol-precursor sterol shown to be an indicator of cholesterol synthesis,<sup>17</sup> and plant sterols, which are indicators of cholesterol absorption,<sup>18,19</sup> were determined in fasting blood samples obtained from subjects in the boiled-coffee group on days 12, 17, 89, 93, and 96. Serum total plant sterols, campesterol and  $\beta$ -sitosterol, and total lathosterol were quantified by gas-liquid chromatography by use of a Packard Model 438S chromatograph with a 25-m×0.25-mm capillary column coated with CP-Sil-5CB (Chrompack, Middelburg, The Netherlands).<sup>17</sup> Nonsaponifiable lipids were converted into their trimethylsilyl ethers by use of Deriva-Sil (Chrompack) after addition of 5- $\alpha$ -cholestane as an internal standard.

## Results

### Compliance

Ninety-eight percent of the packages of coffee, tea, paper filters, fruit juice, mineral water, and cookies

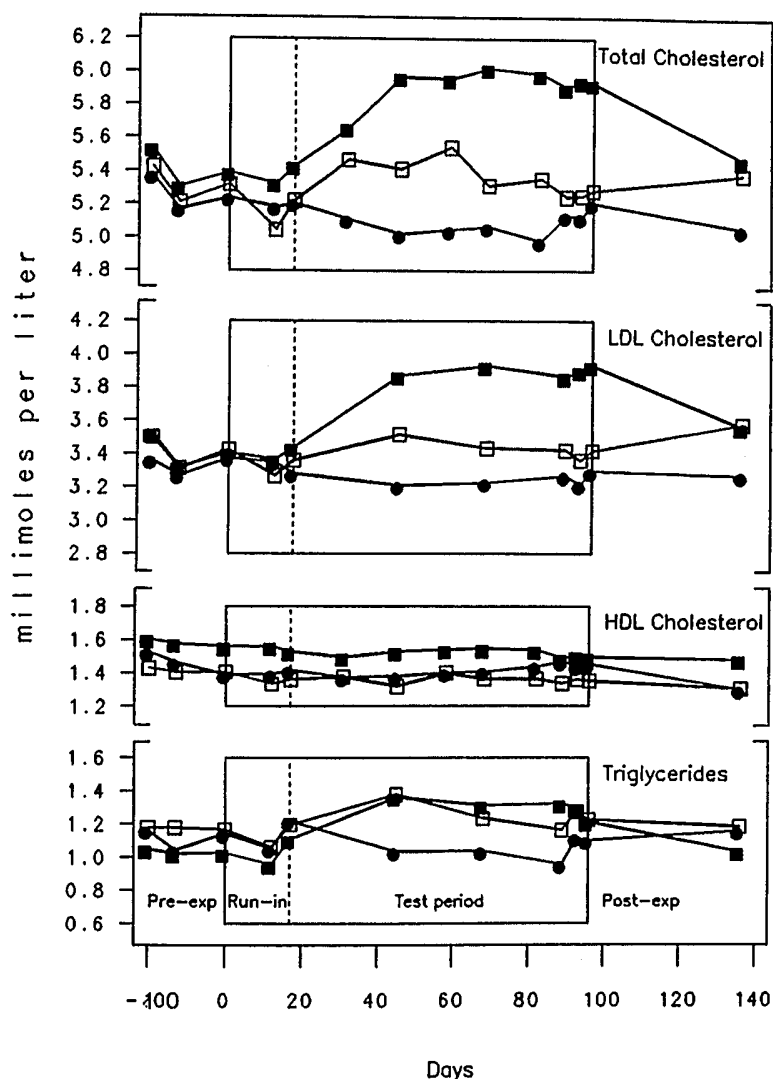


FIGURE 1. Line plots of mean serum concentrations of total (upper panel), low density lipoprotein (LDL, upper middle panel), and high density lipoprotein (HDL, lower middle panel) cholesterol and triglycerides (lower panel) before (Pre-exp), during (Run-in, Test period), and after (Post-exp) the experiment. All 64 subjects drank six cups of boiled and filtered coffee during the run-in period. For the next 79 days, 21 subjects continued consuming the boiled and filtered coffee (□), 22 subjects drank six cups of boiled coffee per day (■), and 21 subjects abstained from coffee (●).

were returned empty by the subjects. During the test period, the mean ( $\pm$ SD) daytime serum caffeine level was  $4.9 \pm 2.2$  mg/l (range, 2.2–11.1 mg/l) in the boiled-coffee group,  $4.7 \pm 2.4$  mg/l (range, 1.2–9.4 mg/l) in the boiled-and-filtered-coffee group, and  $0.4 \pm 1.1$  mg/l (range, 0.1–5.2 mg/l) in the group that drank no coffee. One woman in this group had serum caffeine levels that ranged from 4.4 to 6.2 mg/l (mean, 5.2 mg/l). When her data were eliminated, the mean serum caffeine level in the no-coffee group was  $0.1 \pm 0.1$  mg/l (range, 0.1–0.3 mg/l). In the boiled-and-filtered-coffee group, one man had employed a faulty method to prepare the coffee, and one woman was admitted to a hospital immediately after the trial ended because of poor health, which was unrelated to her participation in the trial. All analyses were performed both with and without the data from these three subjects.

Table 2 shows the changes in body weight and diet from the end of the run-in to the end of the test periods. The changes in body weight and diet did not differ significantly among groups, with the exception of energy intake. Weight changes during the 11.5-

week period ranged from  $-1.9$  to  $2.5$  kg in the boiled-coffee group, from  $-6.3$  to  $3.0$  kg in the group drinking boiled and filtered coffee, and from  $-2.9$  to  $5.6$  kg in the no-coffee group.

#### Lipid Content of Coffee

The boiled and the boiled and filtered coffee was freeze dried, and the lipid content was extracted by the Soxhlet method for 8 hours with petroleum ether (Merck No. 1775, Darmstadt, F.R.G., boiling range,  $40-60^\circ\text{C}$ ). The lipid content of boiled-type coffee prepared with 50 g of ground coffee beans per liter was  $1.98 \pm 0.50$  g/l ( $n=16$ ). However, part of the lipid in this brew was attached to or contained in the floating fine particles of grounds that were not consumed by the subjects. When the boiled-type coffee was carefully decanted into a second Thermos flask after preparation, most of the coffee grounds stayed behind in the first Thermos bottle. The lipid content of this boiled-type coffee beverage, as it was consumed by the subjects, was  $1.00 \pm 0.15$  g/l ( $n=7$ ). For the boiled and filtered coffee, the lipid content was  $0.02 \pm 0.01$  g/l ( $n=14$ ). The paper filter was found to

TABLE 3. Effects of Consuming Boiled Coffee, Boiled and Filtered Coffee, and No Coffee on Fasting Serum Lipid and Lipoprotein Levels

| Variable                   | Boiled coffee<br>(n=22) | Boiled and<br>filtered coffee<br>(n=21) | No coffee<br>(n=21) |
|----------------------------|-------------------------|---|---------------------|
| Total cholesterol (mmol/l) |                         |   |                     |
| Run-in period              | 5.37±0.69               | 5.13±0.89                               | 5.19±0.76           |
| Test period                | 5.92±0.92               | 5.26±0.92                               | 5.15±0.77           |
| Change                     | 0.55±0.52*              | 0.13±0.43                               | -0.04±0.47          |
| LDL cholesterol (mmol/l)   |                         |   |                     |
| Run-in period              | 3.40±0.70               | 3.31±0.84                               | 3.31±0.76           |
| Test period                | 3.90±0.95               | 3.40±0.93                               | 3.26±0.73           |
| Change                     | 0.50±0.46*              | 0.09±0.39                               | -0.06±0.40          |
| HDL cholesterol (mmol/l)   |                         |   |                     |
| Run-in period              | 1.54±0.42               | 1.35±0.28                               | 1.40±0.35           |
| Test period                | 1.49±0.35               | 1.35±0.32                               | 1.45±0.31           |
| Change                     | -0.05±0.18              | 0.00±0.14                               | 0.05±0.15           |
| Triglycerides (mmol/l)     |                         |   |                     |
| Run-in period              | 1.03±0.27               | 1.13±0.34                               | 1.13±0.36           |
| Test period                | 1.28±0.46               | 1.22±0.50                               | 1.05±0.35           |
| Change                     | 0.25±0.31               | 0.09±0.38                               | -0.08±0.21          |
| Apolipoprotein A-I (mg/dl) |                         |   |                     |
| Run-in period              | 129.0±15.9              | 119.3±14.7                              | 122.1±16.6          |
| Test period                | 128.5±15.4              | 117.1±14.4                              | 124.0±14.9          |
| Change                     | -0.5±6.9                | -1.6±7.5                                | 2.0±8.2             |
| Apolipoprotein B (mg/dl)   |                         |   |                     |
| Run-in period              | 82.1±12.2               | 80.9±17.0                               | 82.5±15.8           |
| Test period                | 93.1±19.8               | 83.3±18.3                               | 81.9±15.8           |
| Change                     | 10.9±10.6*              | 2.3±5.8                                 | -0.6±6.8            |

Values are mean±SD. Values were recorded for the 11.5-week period from the end of the run-in period to the end of the test period. To convert from millimoles per liter to milligrams per deciliter, multiply cholesterol values by 38.67 and triglyceride values by 88.54.

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

\*Significant difference from the group drinking boiled and filtered coffee at  $p<0.025$ .

retain 88% of the lipid present in the boiled-type coffee brew before filtration (J. Lebbink, I. Lobbezoo, unpublished data). Varying the drawing time between 2 and 20 minutes or the time that the coffee beverage was stored in the second Thermos bottle (between 30 and 180 minutes) did not influence the amount of lipid in boiled-type or boiled and filtered coffee. The lipid content of boiled-type coffee increased linearly with the amount of ground coffee beans used for preparation: boiled-type coffee prepared with 25, 50, or 75 g of ground beans per liter contained 0.85, 2.10, and 3.28 g lipid/l, respectively.

#### Serum Lipoproteins

The courses of serum lipid levels over time are shown in Figure 1, and the values at the end of the run-in and test periods are shown in Table 3. In the group drinking boiled coffee, the serum total cholesterol concentration rose, on average, by 0.42 mmol/l (16 mg/dl; 95% confidence interval [CI] for difference with the boiled and filtered-coffee group, 0.14–0.71,  $p=0.005$ ) and LDL cholesterol by 0.41 mmol/l (16 mg/dl; 95% CI, 0.16–0.66,  $p=0.002$ ) in comparison with the group drinking boiled and filtered

coffee. The differences in effect on total and LDL cholesterol concentrations between the boiled and the boiled and filtered coffee-consuming groups were present from day 45 of the trial onward, which implies that the cholesterol-raising effect of boiled-type coffee stabilized within 4 weeks. Responses of the mean total and LDL cholesterol concentrations in the no-coffee group did not differ significantly from those in the boiled and filtered-coffee group (Table 3). HDL cholesterol fell slightly by 0.05 mmol/l (2 mg/dl) in the boiled-coffee group relative to the boiled and filtered-coffee group, and it increased by 0.05 mmol/l (2 mg/dl) in the group in which all coffee was eliminated, but the differences among groups were not significant ( $p>0.05$ ). Triglycerides rose slightly by 0.15 mmol/l (13 mg/dl; 95% CI, -0.03 to 0.33,  $p=0.11$ ) in the boiled-coffee group and fell by 0.17 mmol/l (15 mg/dl; 95% CI, -0.36 to 0.02;  $p=0.07$ ) after total elimination of coffee compared with the group that received boiled and filtered coffee throughout the study. There was a significant difference in the response of serum triglycerides between the boiled-coffee and the no-coffee group.

TABLE 4. Effects of Consuming Boiled Coffee on Serum Sterol Levels (n=22)

| Parameter     | Sterol type  |   |   |   |
|---------------|--|---|---|---|
|               | Lathosterol/<br>cholesterol<br>( $\mu\text{mol}/\text{mmol}$ ) | Lathosterol<br>( $\mu\text{mol}/\text{l}$ ) | Campesterol<br>( $\mu\text{mol}/\text{l}$ ) | $\beta$ -Sitosterol<br>( $\mu\text{mol}/\text{l}$ ) |
| Run-in period | 1.00 $\pm$ 0.27  | 5.45 $\pm$ 1.56                             | 14.36 $\pm$ 3.35                            | 8.52 $\pm$ 2.23                                     |
| Test period   | 1.01 $\pm$ 0.33  | 6.07 $\pm$ 1.97                             | 14.87 $\pm$ 4.66                            | 9.48 $\pm$ 2.96                                     |
| Change        | 0.01 $\pm$ 0.18  | 0.62*†                                      | 0.51*                                       | 0.96*‡  |
| Range         | -0.27 to 0.42  | -0.91 to 2.87                               | -3.95 to 9.70                               | -2.12 to 5.32                                       |

Values are mean $\pm$ SD. Values were recorded for the 11.5-week period from the end of the run-in period to the end of the test period.

\*Variable not normally distributed.

†Significant change at  $p < 0.05$ . ‡Significant change at  $p < 0.01$ .

The changes in the levels of apolipoproteins A-I and B in the three groups mirrored those in the levels of HDL and LDL cholesterol, respectively. Responses of apolipoprotein A-I did not differ among the three groups. The mean apolipoprotein B concentration was significantly increased by 8.6 mg/dl (95% CI, 3.8–13.4;  $p=0.001$ ) in the boiled-coffee group relative to the boiled and filtered-coffee group. The ratio of LDL to HDL cholesterol increased from 2.36 to 2.80 in the boiled-coffee group; this rise differed significantly from the change in the LDL to HDL cholesterol ratio in the boiled and filtered-coffee group (95% CI of difference, 0.05–0.60;  $p=0.024$ ). The ratio of apolipoprotein A-I to apolipoprotein B fell by 0.16 in the boiled-coffee group and by only 0.05 in the boiled and filtered-coffee group (95% CI for the difference between groups, -0.19 to -0.03;  $p=0.009$ ).

Within each group, the changes in the levels of serum lipids were similar for men and women. The responses of total and LDL cholesterol levels to treatment were unrelated to the intrinsic serum lipid levels.

Exclusion of the data for three subjects who had been ill or noncompliant (see "Methods") did not affect the results. Likewise, adjustment for changes in body weight and for changes in the reported intake of saturated and polyunsaturated fat and dietary cholesterol had very little influence on the results.

After the study had ended, subjects resumed their habitual consumption of drip-filtered coffee, and their serum cholesterol levels returned to preexperimental levels (Figure 1).

#### Serum Lathosterol and Plant Sterols

As a first approach to the mechanism of action by which boiled coffee raises cholesterol levels, changes in serum lathosterol and plant sterol levels were measured in subjects drinking boiled coffee. Table 4 lists the mean serum concentrations of total lathosterol, lathosterol to cholesterol ratio, campesterol, and  $\beta$ -sitosterol for the 22 subjects in the boiled-coffee group at the end of the run-in period (when they consumed boiled and filtered coffee) and at the end of the test period (when they consumed boiled coffee). The mean values for serum sterols are in

agreement with those reported previously.<sup>17,18</sup> With boiled coffee, the serum level of the cholesterol precursor lathosterol was significantly increased by 11%, but the lathosterol to cholesterol ratio did not change. Serum campesterol level remained constant, but mean serum  $\beta$ -sitosterol increased by 11%.

## Discussion

### Role of Paper Filters

Our findings show that the paper filter plays a crucial role in eliminating the hypercholesterolemic factor from coffee. The cholesterol-elevating effect of boiled-type coffee disappeared when the liquid was poured through a paper filter before consumption. Consumption of this boiled and filtered coffee had no effect on serum cholesterol levels when compared with effects from drinking no coffee at all. Compliance was very high: 98% of the 20,866 packages distributed were returned by the subjects, and mean serum caffeine levels were high in the coffee-drinking groups and, except for one subject, were close to zero in the group that was given fruit juice and mineral water. After adjustment for dietary changes, the mean rise in serum total cholesterol level was 0.49 mmol/l or 9% in the group drinking boiled-type coffee, which is similar to the cholesterol-raising effect of boiled coffee in other studies. Thus, eight cups of boiled coffee raised cholesterol by 0.89 mmol/l (10%) in the study of Aro et al,<sup>5</sup> and four to six cups of boiled-type coffee caused a rise of 0.48 mmol/l (10%) in the study of Bak and Grobbee.<sup>6</sup> The absence of an effect on serum lipid levels produced by abstaining from filtered coffee is in agreement with previous findings.<sup>4–6,8,20</sup>

### Role of Coffee Lipid

A number of explanations have been advanced to explain the effect of boiled coffee on serum cholesterol levels; these include the length of time that the coffee grounds are in hot water<sup>1,6</sup> and the hardness<sup>21</sup> and temperature<sup>6</sup> of the water. Bak et al<sup>6</sup> showed that boiled-type coffee prepared in a Thermos flask at about 93°C has the same effect on serum lipids as coffee boiled on a stove at 100°C in the original Scandinavian way. The water in an electric coffee

maker dripping onto a paper filter cone containing the ground beans averages 90–95°C, but the temperature of the sediment quickly decreases to 87.5°C. Thus, the temperature of the water during preparation of boiled or boiled-type coffee is indeed higher than that during the preparation of drip-filtered coffee, but this does not explain why such coffee raises cholesterol levels. Recently, we<sup>10</sup> showed that the lipid-rich supernatant of boiled coffee obtained after centrifugation raised serum cholesterol levels by 23%, and we suggested that the cholesterol-elevating factor is contained in the lipid. The present study now shows that boiled-type coffee loses both its lipids and its cholesterol-raising properties after filtration through a paper filter and that the lipid is retained in the paper filter. Thus, the responsible factor probably is contained in the coffee lipid. Since dietary triglycerides and phospholipids do not raise serum cholesterol when eaten in amounts of 1 g/day, we speculate that the cholesterol-raising factor makes up part of the sterol-rich fraction of the lipid.

#### Time Course

Previous studies have not clarified how long it takes for the effect of boiled coffee to express itself fully; it was evident, however, that stabilization is slow compared with the effect of various fatty acids and dietary cholesterol on serum lipids, which reach a plateau after 10–14 days.<sup>22</sup> We have now shown that the effect of boiled coffee reaches a plateau after 4 weeks of intervention. This is a remarkably long period, and it should have implications for the mechanism involved.

#### High Density Lipoprotein and Triglycerides

As was shown in other studies, boiled or boiled-type coffee had no effect on HDL and apolipoprotein A-I levels.<sup>5,6</sup> Triglyceride levels showed a tendency to higher values during consumption of boiled coffee and lower values after elimination of coffee (Reference 5 and Table 3), but neither the present nor other studies have produced the dramatic rise of 55% in serum triglycerides seen with purified coffee lipid.<sup>10</sup> It remains to be established whether this is due to a qualitative difference between isolated coffee lipid and boiled coffee or is simply a matter of dosage.

#### Mechanisms

Serum lathosterol was used as a monitor of whole-body synthesis of cholesterol. However, lathosterol concentrations are correlated with the total serum cholesterol level.<sup>17</sup> Differences in serum lathosterol can occur as a mere consequence of differences in the number of acceptor lipoprotein particles in serum.<sup>23</sup> Therefore, we looked at both the absolute serum lathosterol concentration and the lathosterol to cholesterol ratio. The increase of 11% in serum lathosterol in the group drinking boiled coffee was paralleled by a 9% increase in serum cholesterol level. This might suggest that increased synthesis of cholesterol does not play a major role in the elevation of serum cholesterol levels.

Serum campesterol is considered an indicator of the fractional absorption of cholesterol in the gut, while serum  $\beta$ -sitosterol levels are more strongly associated with the intake of linoleic acid.<sup>19</sup> Thus, the absence of an increase in serum campesterol provides tentative evidence that boiled coffee did not cause a major increase in the fractional absorption of dietary cholesterol. Therefore, decreased clearance of VLDL with an increased conversion to LDL or a decreased clearance of LDL cholesterol could provide alternative explanations for the cholesterol-raising effect of boiled coffee. However, our findings do not exclude effects of boiled coffee on lipoprotein synthesis and turnover. This should be explored further in metabolic studies.

#### Conclusions

Our data show that the method used to separate coffee grounds from brew is crucial in determining the hypercholesterolemic potential of coffee beverages. We cannot yet answer the questions whether the temperature of the water or the length of time that coffee grounds are in contact with hot water are also relevant. Thus, we cannot tell whether coffee brewed without a paper filter but with lower-temperature water or a shorter contact time will raise cholesterol levels. It is clear, however, that those concerned about the cholesterol-elevating effect of coffee can safely consume coffee so long as they drink coffee that is filtered through paper.

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