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## Effect of a lipid-rich fraction from boiled coffee on serum cholesterol

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**Scandinavian-style boiled coffee, which raises serum cholesterol, was found to contain more lipid material than drip filter coffee, which does not. Ten volunteers consumed a lipid-enriched fraction from boiled coffee for six weeks: the supplement provided 77 g of water, 1.3 g of lipid, and 1.6 g of other solids per day. Serum cholesterol rose in every subject; the mean rise was 0.74 mmol/l after three weeks (range -0.09 to 1.48 mmol/l) and 1.06 SD 0.37 mmol/l or 23% after six weeks (range 0.48 to 1.52 mmol/l). The increase was mainly due to low-density-lipoprotein cholesterol, which rose by 29%, but very-low-density lipoprotein cholesterol was also raised, as evidenced by a 55% rise in triglycerides. High-density-lipoprotein cholesterol was unchanged. After supplementation had ended, lipid levels returned to baseline. Boiled coffee thus contains a lipid that powerfully raises serum cholesterol.**

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### Introduction

Scandinavians have traditionally made their coffee by boiling ground coffee beans and water in a saucepan, and

decanting the fluid into a cup. In 1983, a study from Norway showed a strongly significant relation between the amount of coffee ingested and serum total cholesterol.<sup>1</sup> Recently it was found that this association is specifically due to boiled coffee.<sup>2</sup> Controlled experiments<sup>3,4</sup> showed that abstaining from boiled coffee caused large decreases in serum cholesterol in healthy volunteers<sup>3</sup> and in hypercholesterolaemic men.<sup>4</sup> In a Finnish trial<sup>5</sup> volunteers showed a rise in cholesterol of 0.64 mmol/l after four weeks of consumption of 8 cups of boiled coffee per day but a fall after consumption of regular drip filter coffee or tea. A similar cholesterol-raising effect of boiled but not of drip coffee was found in a Dutch experiment.<sup>6</sup> A switch from boiled to drip coffee consumption is believed to have contributed to the fall in serum cholesterol and coronary heart disease observed in Finland in the past 20 years.<sup>2</sup> However, it is totally unclear why boiled coffee should cause hypercholesterolaemia. Nor do we know to what extent other types of coffee such as Turkish, Greek, or espresso

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coffee will raise cholesterol. A report that decaffeinated coffee raises cholesterol may also indicate that the relevance of the putative hypercholesterolaemic factor extends beyond Scandinavia.<sup>7</sup>

Evidently the effect is caused by a factor present in boiled but not in drip filter coffee. We noticed that boiled coffee, when centrifuged, contains obvious lipid material on the surface. The lipid content of boiled coffee turned out to be 0.1–0.2 g/dl of brew as opposed to about 0.001 g/dl for drip filter coffee, made by pouring hot water on ground beans in a paper filter cone and allowing the brew to drip into a cup or pot. To investigate whether the cholesterol-raising effect of boiled coffee was due to its lipid content we prepared a lipid-enriched fraction from boiled coffee and administered it to healthy volunteers.

### Materials and methods

1350 kg of water was heated to boiling in 150 kg batches in a steel kettle with 15 kg of coarsely ground coffee per batch. We used a regular commercial Dutch coffee for institutional use, consisting largely of arabica beans with a small amount of robusta beans. The grounds were allowed to settle and the brew (mean 108.5 kg per batch) was siphoned out.

The hot brew was centrifuged in a Sharples continuous-flow centrifuge (Rueil-Malmaison, France) at 13 200 g. The denser fluid was allowed to run off, and 4.5 kg of lipid-enriched supernatant per run was collected. It contained 96.4 g of water and 3.6 g of dry matter per 100 g. Out of this 3.6 g, 1.64 SD 0.67 g was lipid (as determined by extraction with petroleum ether). Of this lipid, 0.15 g was unsaponifiable—ie, it was not hydrolysed by boiling for 30 min in ethanolic KOH.<sup>8</sup> The recovery of lipid from the original brew was 41% and the enrichment 10 times compared with boiled coffee. If lipid recovery was 41%, 80 g of lipid-rich fraction corresponded to 108 g of ground coffee. Lipid was not detectable in the denser effluent. The mean caffeine content was 136 (SD 15) mg/100 g in the lipid-rich supernatant and 162 (26) mg/100 g in the dense effluent.

80 g/day of the lipid-rich supernatant, supplying a mean of 1.3 g of coffee lipids per day, was given to five men and five women for six weeks. Their mean age was 28 (SD 6) years and their body mass index (weight/height<sup>2</sup>) 22.0 (1.8) kg/m<sup>2</sup>. Two men smoked. All subjects were in good health, none had glucosuria or proteinuria, and none was taking any medication known to affect serum lipids. Participants habitually consumed up to five cups of regular drip filter coffee a day. The protocol and aim of the study were explained to the subjects, who gave their informed consent. The study was approved by the human ethics committee of the department.

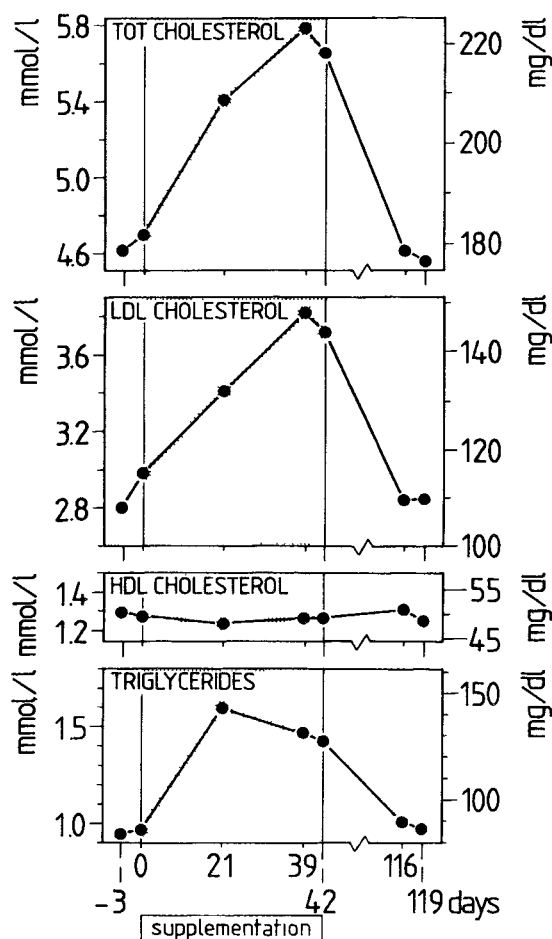
Participants replaced their habitual desserts by experimental desserts made from 80 g of lipid-rich supernatant and 200 g of

TABLE 1—MEAN DAILY INTAKE OF ENERGY AND NUTRIENTS OF SUBJECTS JUST BEFORE THE TRIAL, AT WEEK SIX OF THE SUPPLEMENTATION PERIOD, AND 75 DAYS AFTER COMPLETION OF THE TRIAL

	Pre-trial (wk 0)	End of trial (wk 6)	Post-trial (wk 17)
Energy (kcal/day)	2550 (810)	2450 (690)	2880 (810)
Protein (% of calories)	14 (4)	13 (4)	13 (1)
Fat (% of calories):	33 (7)	30 (8)	36 (7)
Saturated fatty acids	12 (4)	12 (4)	14 (3)
Monounsaturated fatty acids	12 (3)	11 (4)	13 (3)
Polyunsaturated fatty acids	7 (3)	6 (2)	7 (3)
Carbohydrates (% of calories)	50 (4)	51 (6)	47 (8)
Alcohol (% of calories)	4 (5)	6 (6)	3 (3)
Cholesterol (mg/day)	230 (145)	225 (150)	260 (95)
Dietary fibre (g/day)	35 (10)	30 (8)	36 (10)
Keys score (mmol/l)	4.9 (0.3)	4.9 (0.3)	5.1 (0.3)

Values for energy and nutrients, mean (SD), were calculated from 24 h recalls with use of the Netherlands Nutrients Data Base

Keys score predicts the combined effects of the intake of saturated and polyunsaturated fatty acids and cholesterol on serum cholesterol concentrations<sup>14</sup>



Mean serum total, LDL, and HDL cholesterol, and triglyceride levels before, during, and after supplementation of lipid-rich coffee fraction.

custard. The custard was made from either whole milk or skim milk according to the subject's usual preference. Assessment of nutrient intake by 24 h recall<sup>9</sup> before the trial, at the end, and eleven weeks after the end showed that the Keys score, which combines the predicted effects of fatty acids and dietary cholesterol on serum cholesterol, was essentially constant (table 1). Over the six week trial period, body weight changed by -0.3 (SD 1.1) kg (range -2.0 to +1.6 kg).

Blood samples were taken after a 12 h fast. All venepunctures were performed by the same technician, in the same location, and at the same time of the same day of the week. Serum was stored at -80°C and analysed enzymatically for total and high-density-lipoprotein (HDL) cholesterol and triglycerides.<sup>10,11</sup> The samples of days -3, 0, 21, 39, and 42 (= week six) were analysed in one run, and samples obtained on days 116 and 119 in a later run. The coefficient of variation within runs was 1% for total cholesterol, 2% for HDL cholesterol, and 1% for triglycerides. Mean bias with regard to the target values of three serum pools provided by the Centers for Disease Control, Atlanta, GA, USA, was 0.01 mmol/l for total cholesterol and -0.08 mmol/l for triglycerides. Bias with regard to the target value for HDL cholesterol of three pools of the North West Lipid Research Clinic was -0.06 mmol/l.<sup>12</sup> Low-density-lipoprotein (LDL) cholesterol was calculated.<sup>13</sup>

### Results

All participants completed the trial successfully and none reported any illnesses or changes in lifestyle that might have affected blood lipids. Upon consumption of the lipid-rich coffee fraction, subjects showed a rise in total cholesterol from a mean pre-trial value of 4.66 (SD 0.73) mmol/l to 5.40 (0.84) mmol/l after three weeks and 5.72 (0.93) mmol/l after six weeks (figure). Eleven weeks after completion of the supplementation period, total cholesterol had returned to 4.59 (0.72) mmol/l. The mean rise at six weeks was 1.06

TABLE II—MEAN (SD) SERUM TOTAL, LDL, AND HDL CHOLESTEROL AND TRIGLYCERIDE CONCENTRATIONS AND MEAN (SD) CHANGES OBSERVED DURING AND AFTER SUPPLEMENTATION

	Pre-trial (wk 0)	End of trial (wk 6)	Post-trial (wk 17)	Change after 6 wk	95% CI
Total cholesterol mmol/l	4.66 (0.73)	5.72 (0.93)*	4.59 (0.72)	1.06 (0.37)	0.75 to 1.37
LDL cholesterol mmol/l	2.92 (0.70)	3.78 (0.94)*	2.84 (0.39)	0.85 (0.37)	0.56 to 1.14
HDL cholesterol mmol/l	1.29 (0.27)	1.27 (0.31)	1.29 (0.30)	-0.02 (0.19)	-0.16 to 0.12
Triglycerides mmol/l	0.96 (0.25)	1.46 (0.44)*	1.00 (0.29)	0.51 (0.35)	0.20 to 0.91

\*Significantly different from week 0 and week 17 ( $p < 0.0002$ )

To convert from mmol/l to mg/dl, multiply cholesterol values by 38.67 and triglyceride values by 88.54.

(0.37) mmol/l or 23% (95% confidence interval 0.75 to 1.37 mmol/l,  $p < 0.0001$ ). A rise in cholesterol was seen in each of the ten subjects (range 0.48 to 1.52 mmol/l). LDL cholesterol rose by 0.85 (SD 0.37) mmol/l or 29% ( $p < 0.0001$ ; range 0.32 to 1.32 mmol/l) and total triglycerides by 0.51 (0.35) mmol/l or 55% ( $p = 0.0002$ ; range 0.17 to 1.28 mmol/l). HDL cholesterol concentrations remained constant. After the trial period serum lipoproteins returned to pre-trial values (figure and table II).

### Discussion

We found that one small cup of coffee fluid enriched with 1.3 g of coffee lipid per day caused a rise in LDL cholesterol of 29.1 (SD 9.8)%. Although the trial was not placebo controlled the pronounced and consistent rise in cholesterol and triglycerides seen here is unlikely to be explained by any factor other than the consumption of the lipid-rich coffee fraction. The changes in dietary intake (including the custard) would be expected to have zero effect on serum cholesterol concentration (table I), and laboratory drift was excluded by analysis of all samples of the trial in one run.

Aro et al<sup>5</sup> reported that subjects consuming eight cups of boiled coffee a day for four weeks showed a rise in total cholesterol of 0.89 mmol/l relative to subjects consuming the same amount of drip coffee. Eight cups (about 1 litre) of boiled coffee would provide about 1.5 g of lipid. The 80 ml of lipid-rich coffee fraction consumed by our subjects provided 1.3 g of lipids, and caused a rise in cholesterol of the same order of magnitude as that seen by Aro et al with 1 litre of boiled coffee. Thus the centrifugation process caused an enrichment both in the cholesterol-raising factor and in the amount of lipid by a factor of 10—evidence that the cholesterol-raising factor is contained in the lipid.

Our lipid-rich supernatant provided 109 mg of caffeine a day. This is unlikely to have affected blood lipids, because we have shown in volunteers that consumption of up to 425 mg caffeine a day has no effect on serum lipoproteins.<sup>15</sup> We also think it unlikely that water-soluble substances other than caffeine caused the observed rise in serum lipids. Centrifugation caused a tenfold increase in cholesterol-raising factor in the supernatant, and a biological compound that floats upward upon centrifugation is usually a lipid. In addition we have found that the lipid material from boiled coffee does not pass a paper filter (Zock PL, van Vliet T, unpublished); this may explain why drip coffee lacks a cholesterol-raising effect.

The absence of an effect of the lipid-rich supernatant on HDL is consistent with findings with boiled coffee.<sup>5,6</sup> The striking rise in triglycerides of 55% has not been reported with boiled coffee. It suggests that the primary effect of the coffee lipid fraction could be on VLDL, with LDL rising as a secondary consequence.

About 90% of the lipid material in the lipid-rich supernatant could be hydrolysed by boiling in ethanolic

KOH, and must thus have consisted of triacylglycerols (triglycerides), phospholipids, and other esters of fatty acids. As triglycerides and phospholipids do not increase serum cholesterol when given in amounts of 1 g per day, we speculate that the cholesterol-raising substances must have formed part of the 120 mg of unsaponifiable lipid matter in the supplement. The unsaponifiable lipids in coffee oil are a mixture of sterols, hydrocarbons, squalene, diterpene alcohols, and other, unknown, compounds.<sup>16</sup> If the substance responsible for the cholesterol-raising effect of boiled coffee is indeed located in the 120 mg of unsaponifiables consumed daily by our subjects, then this "coffee lipid factor" must be a powerful natural cholesterol-raising compound. Identification of this substance might help to identify foods that should be avoided by patients with hypercholesterolaemia. In addition it might further our understanding of the regulation of plasma lipid levels.

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