ELSEVIER

Atherosclerosis 132 (1997) 251–254

Rapid communication

The cholesterol-raising diterpenes from coffee beans increase serum lipid transfer protein activity levels in humans

Arie van Tol [a,*], Rob Urgert [b], Ruth de Jong-Caesar [a], Teus van Gent [a], Leo M. Scheek [a], Baukje de Roos [b], Martijn B. Katan [b]

[a] *Department of Biochemistry, Cardiovascular Research Institute (COEUR), Faculty of Medicine and Health Sciences, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, Netherlands*

[b] *Wageningen Agricultural University, Department of Human Nutrition, P.O. Box 8129, 6700 EV Wageningen, Netherlands*

Received 8 November 1996; received in revised form 10 February 1997; accepted 11 April 1997

Abstract

Cafestol and kahweol–diterpenes present in unfiltered coffee— strongly raise serum VLDL and LDL cholesterol and slightly reduce HDL cholesterol in humans. The mechanism of action is unknown. We determined whether the coffee diterpenes may affect lipoprotein metabolism via effects on lipid transfer proteins and lecithin:cholesterol acyltransferase in a randomized, double-blind cross-over study with 10 healthy male volunteers. Either cafestol (61–64 mg/day) or a mixture of cafestol (60 mg/day) and kahweol (48–54 mg/day) was given for 28 days. Serum activity levels of cholesterylester transfer protein, phospholipid transfer protein and lecithin:cholesterol acyltransferase were measured using exogenous substrate assays. Relative to baseline values, cafestol raised the mean ($\pm$S.D.) activity of cholesterylester transfer protein by $18 \pm 12\%$ and of phospholipid transfer protein by $21 \pm 14\%$ (both $P < 0.001$). Relative to cafestol alone, kahweol had no significant additional effects. Lecithin:cholesterol acyltransferase activity was reduced by $11 \pm 12\%$ by cafestol plus kahweol ($P = 0.02$). It is concluded that the effects of coffee diterpenes on plasma lipoproteins may be connected with changes in serum activity levels of lipid transfer proteins. © 1997 Elsevier Science Ireland Ltd.

Keywords: Cafestol; CETP; Coffee; Diterpenes; Kahweol; LCAT; Lipid transfer proteins; PLTP

1. Introduction

The cholesterol-raising effect of unfiltered, boiled coffee as consumed, for example, in Turkey and in some Scandinavian countries has long been controversial [1]. Recently, it was shown that the diterpenes cafestol and kahweol are responsible for this effect [2,3]. Cafestol and kahweol are very powerful compounds; each 10 mg of cafestol—the amount present in three cups of unfiltered coffee such as Turkish and Scandinavian boiled, or French press (also called cafetiere) coffee [4]—ingested per day increases serum cholesterol concentrations by about 0.13 mmol/l [2]. The effect of coffee diterpenes on total cholesterol is due to raised LDL and VLDL cholesterol and decreased HDL cholesterol is also observed [2,5–7]. These changes are indicative of increased risk for atherosclerosis and coronary artery disease.

The mechanism by which coffee diterpenes influence lipid metabolism in the human body is unknown. It has been suggested that coffee diterpenes (or some of their metabolites) could lead to an overloading of LDL particles with cholesterol, possibly mediated by increased serum concentrations of cholesterylester transfer protein (CETP) [8]. This protein catalyzes the

* Corresponding author. Tel.: +31 10 4087321; fax: +31 10 4360615.

PII S0021-9150(97)00099-3

transfer of plasma cholesterylesters, synthesized by lecithin:cholesterol acyltransferase (LCAT) from HDL to apolipoprotein B-containing lipoproteins: LDL and VLDL [9]. Phospholipid transfer protein (PLTP) may assist in this process [10] because of its ability to convert HDL into larger and smaller particles [11]. We investigated whether serum lipid transfer proteins (CETP as well as PLTP) and LCAT activity are affected during the induction of high serum VLDL + LDL cholesterol and low HDL cholesterol concentrations by coffee diterpenes in man.

2. Methods

2.1. Preparation of diterpenes

As it was practically impossible to prepare pure kahweol in sufficient quantity, we decided to compare the effects of a mixture of cafestol and kahweol relative to cafestol alone. Diterpenes were purified from coffee oil provided by Nestec, Switzerland. They were given as palmitate esters dissolved in oil (sunflower plus palm oil, w/w 3:2). Purities of the diterpenes ranged from 92.2 to 99.7%; impurities consisted of free cafestol, kahweol, palmitic acid and cafestol and kahweol dipalmitate.

2.2. Subjects

Approval for the experiment was obtained from the Human Ethics Committee of the department and from the Nijmegen University Hospital Ethical Committee. Ten male volunteers were recruited. The study protocol was explained and written informed consent was obtained. All subjects were in good health. None was taking medication known to affect serum lipids or serum liver enzymes. The mean age was 24 ± 4 (years $\pm$ S.D.) and the mean body mass index was 21 ± 2 (kg/m^2 $\pm$ S.D). Two of the subjects were smokers. The mean use of alcohol-containing beverages was less than 1 beverage per day.

2.3. Design

Subjects were randomly allocated to one of two supplementation sequences after being grouped in pairs with similar cholesterol level. During the first intervention period of 28 days, half of the subjects were given five capsules per day which provided a total of 64 mg cafestol and 1 mg kahweol (amounts expressed as free alcohols) dissolved in 2 g oil. The other five subjects received 60 mg cafestol plus 54 mg kahweol per day dissolved in the oil mixture. Supplements were switched in the second intervention period of 28 days; analyzed dosages of cafestol and kahweol were now 61 and 0 mg for pure cafestol, and 60 and 48 mg for the mixture. Subjects took two capsules at breakfast and three with their evening meals. There was a nine weeks wash-out period between the two interventions. Subjects as well as investigators were blinded to the supplement sequences.

Subjects maintained their usual dietary and living habits, abstained from coffee types other than paper-filtered or instant (soluble) coffee, and restricted alcohol use to a maximum of twenty alcohol-containing beverages per week (checked by daily records). Three subjects (2 in the first and 1 in the second experimental period) were switched to placebo halfway through the treatment period, because of elevated serum activities of alanine aminotransferase. These subjects turned out to have been on cafestol plus kahweol. The values obtained after 2 weeks of intervention were used instead of the final values.

2.4. Blood sampling and assays

Serum was obtained after an overnight fast and stored at -80°C. CETP activity levels were assayed after removal of endogenous VLDL + LDL [12,13]. The CETP activity obtained by this method is strongly correlated with CETP mass [14]. PLTP activity levels were assayed using a phospholipid vesicles-HDL system. This method is specific for PLTP activity and the phospholipid transfer promoting properties of CETP do not interfere with the assay [13]. LCAT activity levels were determined using excess exogenous substrate containing [^{3}H]cholesterol [15].

All assays of CETP, PLTP and LCAT were carried out in duplicate and the serum samples were assayed in one run, using single batches of substrates. The within assay coefficients of variation were 2.7, 3.5 and 4.5%, respectively. The activities are expressed as arbitrary units, relative to the activity in a human poolserum (percent reference serum). LCAT, PLTP and CETP activities are stable in serum stored at -80°C.

2.5. Statistics

The effects of cafestol were analyzed by subtracting baseline values from values after cafestol supplementation and the effects of the mixture relative to cafestol alone by subtracting the values after cafestol alone from those after ingestion of cafestol plus kahweol. All variables were normally distributed. Differences were tested against zero with two-sided paired t-tests.

3. Results

Treatment with cafestol alone increased serum activities (mean $\pm$ S.D.) of CETP by 18 ± 12%, and those of

Table 1
Mean serum activities of CETP, LCAT and PLTP in 10 healthy male volunteers after consumption of 61–64 mg/day of cafestol alone or of a mixture of 60 mg/day of cafestol plus 48–54 mg/day of kahweol for 28 days each in a cross-over study

	Cafestol treatment[a,b]				Cafestol + Kahweol treatment[a,b]				Cafestol + kahweol minus cafestol	
	Pre-treatment	Post-treatment	Change	*P*	Pre-treatment	Post-treatment	Change	*P*	Difference (95% CI)	*P*
CETP	101 ± 26	119 ± 24	18 ± 12	<0.001	103 ± 22	119 ± 30	16 ± 14	0.007	0 (−12–13)	0.96
PLTP	101 ± 18	121 ± 17	21 ± 14	<0.001	102 ± 26	126 ± 18	24 ± 16	<0.001	4 (−6–15)	0.40
LCAT	84 ± 11	82 ± 9	−2 ± 5	0.18	91 ± 10	80 ± 11	−11 ± 12	0.02	−2 (−9–4)	0.50

[a] All values are expressed as percent of the activity in reference human poolserum; [b] values are means ± S.D.

PLTP by 21 ± 14% (both $P < 0.001$, see Table 1 and Fig. 1). The presence of kahweol in the mixture had little additional effect on the lipid transfer protein activities over cafestol alone. LCAT activity levels were not changed upon cafestol treatment, but were reduced significantly upon intake of the mixture. The pre-treatment activities of CETP, PLTP or LCAT obtained just before the two separate intervention periods were not different, showing the absence of carry-over effects.

4. Discussion

The major finding is that coffee diterpenes may have an effect on the activity levels of the lipid transfer proteins CETP and PLTP and the enzyme LCAT, in addition to or via the effects on plasma lipoproteins. In the present study, consumption of cafestol alone raised serum LDL cholesterol by 19% and reduced HDL cholesterol by 4%, while consumption of a mixture of cafestol and kahweol raised LDL cholesterol by 23% and reduced HDL by 6% relative to baseline [16].

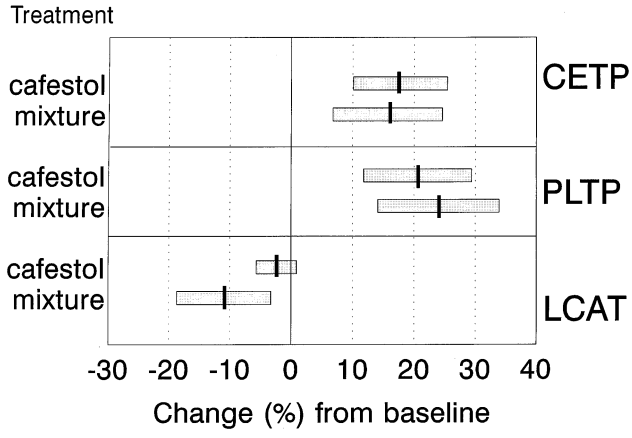

Fig. 1. Mean changes (with 95% confidence intervals) in activities of CETP, PLTP and LCAT after ingestion of either 61–64 mg/day of cafestol alone or of a mixture of 60 mg/day of cafestol plus 48–54 mg/day of kahweol for 28 days each in a cross-over study with 10 healthy male volunteers.

An increase in CETP activity is expected to lower HDL cholesterol and may contribute to an increase in VLDL and LDL cholesterol. This is exactly what is seen in subjects who drink boiled, unfiltered coffee [2] or take diterpenes by capsule as in the present experiment. As apolipoprotein concentrations were not measured in our subjects we cannot distinguish at present between changes in number of lipoprotein particles or cholesterol content per particle. Elevated CETP levels may be a cause as well as a consequence of high plasma LDL cholesterol concentrations. Data from earlier animal experiments show that i.v. injection of purified human CETP into rats, a species without endogenous CETP activity, results in increased plasma apo B and LDL cholesterol concentrations [17]. On the other hand, Masucci-Magoulas et al. [18] found that knock-out of the LDL receptor gene in transgenic mice expressing human CETP not only causes an increase in plasma LDL, but also elevation of plasma CETP levels. These data show that high plasma cholesterol is able to cause an increase in plasma CETP by induction of CETP gene expression.

The present study shows parallel effects on CETP and PLTP. The exact physiological function of PLTP is unknown. There is evidence that PLTP may assist in CETP-mediated lipid transfer [19], possibly by its ability to convert HDL into larger and smaller particles [11]. Our experiments do not allow conclusions to be drawn about putative gene regulation by diterpenes, but it is noteworthy that the overall structures of the CETP and PLTP genes are quite similar [19]. Animal experiments show that both CETP and PLTP are up-regulated by cholesterol feeding, suggesting cholesterol-responsive elements in both genes [20,21].

The effect of coffee diterpenes on serum lipoproteins appears to be linear up to doses of 200 mg [4]. The addition of kahweol had little extra effect on serum activities of CETP and PLTP, suggesting that cafestol is the main factor affecting CETP and PLTP. However, the mixture of cafestol and kahweol was necessary for a significant effect on LCAT activity. As the liver is the major organ for LCAT synthesis, the small decrease in LCAT activity levels by the mixture of cafestol and kahweol could be caused by a slightly impaired integrity of liver cells at high diterpene intake [16].

The amounts of diterpenes supplied per day were high, e.g. equivalent to those present in 10–20 cups of boiled Turkish or French press coffee [4]. As yet it is unknown whether smaller doses could also affect plasma lipid transfer proteins. However, the present data provide a mechanism by which coffee diterpenes could influence serum lipoprotein metabolism.

Acknowledgements

This study was supported by the Netherlands Heart Foundation through grant No. 900-562-091 of the Netherlands Organization of Scientific Research (NWO), plus supplemental funding by the Institute for Scientific Information on Coffee. We thank the volunteers for their participation and enthusiasm, our colleagues at Nestec Research Center, Switzerland for advice and materials, Joanna Kruimel, MD, for medical supervision, laboratory personnel for blood sampling and measurement, Truus Kosmeijer-Schuil and Guido van der Weg for preparation of the treatments and Natasja Essed for assistance with the study.

References

[1] Thelle DS, Arnesen E, Förde OH. The Tromsö Heart Study. Does coffee raise serum cholesterol?. N Engl J Med 1983;308:1454–7.

[2] Weusten-van der Wouw MPME, Katan MB, Viani R, et al. Identity of the cholesterol-raising factor from boiled coffee and its effects on liver function enzymes. J Lipid Res 1994;35:721–33.

[3] Heckers H, Gobel U, Kleppel U. End of the coffee mystery: diterpene alcohols raise serum low-density lipoprotein cholesterol and triglyceride levels. J Intern Med 1994;235:192–3.

[4] Urgert R, Van der Weg G, Kosmeijer-Schuil TG, Van de Bovenkamp P, Hovenier R, Katan MB. Levels of the cholesterol-elevating diterpenes cafestol and kahweol in various coffee beans. J Agric Food Chem 1995;43:2167–72.

[5] Zock PL, Katan MB, Merkus MP, Van Dusseldorp M, Harry-van JL. Effect of a lipid-rich fraction from boiled coffee on serum cholesterol. Lancet 1990;335:1235–7.

[6] Mensink RP, Lebbink WJ, Lobbezoo IE, Weusten-van der Wouw MPME, Zock PL, Katan MB. Diterpene composition of oils from Arabica and Robusta coffee beans and their effects on serum lipids in man. J Intern Med 1995;237:543–50.

[7] Van Rooij J, Van der Stegen GHD, Schoemaker RC, et al. A placebo-controlled parallel study of the effect of two types of coffee oil on serum lipids and transaminases: identification of chemical substances involved in the cholesterol-raising effect of coffee. Am J Clin Nutr 1995;61:1277–83.

[8] Grundy SM. Cholesterol-raising effects of coffee: clues to regulation of cholesterol metabolism. J Intern Med 1995;238:475–7.

[9] Tall AR. Plasma cholesteryl ester transfer protein. J Lipid Res 1993;34:1255–74.

[10] Tollefson JH, Ravnik S, Albers JJ. Isolation and characterization of a phospholipid transfer protein [LTP-II] from human plasma. J Lipid Res 1988;29:1593–602.

[11] Jauhiainen M, Metso J, Pahlman R, Blomqvist S, Van Tol A, Ehnholm C. Human plasma phospholipid transfer protein causes high density lipoprotein conversion. J Biol Chem 1993;268:4032–6.

[12] Groener JEM, Pelton RW, Kostner GM. Improved estimation of cholesteryl ester transfer/exchange activity in serum or plasma. Clin Chem 1986;32:283–6.

[13] Speijer H, Groener JE, van Ramshorst E, Van Tol A. Different locations of cholesteryl ester transfer protein and phospholipid transfer protein activities in plasma. Atherosclerosis 1991;90:159–68.

[14] Hannuksela M, Marcel YL, Kesaniemi YA, Savolainen MJ. Reduction in the concentration and activity of plasma cholesteryl ester transfer protein by alcohol. J Lipid Res 1992;33:737–44.

[15] Glomset JA, Wright JL. Some properties of a cholesterol esterifying enzyme in human plasma. Biochim Biophys Acta 1964;89:266–7.

[16] Urgert R, Essed N, van der Weg G, Kosmeijer-Schuil TG, Katan MB. Separate effects of the coffee diterpenes cafestol and kahweol on serum lipids and liver aminotransferases. Am J Clin Nutr 1997;65:519–24.

[17] Groener JEM, Van Gent T, Van Tol A. Effect of lipid transfer protein on plasma lipids, apolipoproteins and metabolism of high-density lipoprotein cholesteryl ester in the rat. Biochim Biophys Acta 1989;1002:93–100.

[18] Masucci-Magoulas L, Plump A, Jiang XC, Walsh A, Breslow JL, Tall AR. Profound induction of hepatic cholesteryl ester transfer protein transgene expression in apolipoprotein E and low density lipoprotein receptor gene knockout mice-A novel mechanism signals changes in plasma cholesterol levels. J Clin Invest 1996;97:154–61.

[19] Albers JJ, Wolfbauer G, Cheung MC, et al. Functional expression of human and mouse plasma phospholipid transfer protein: effect of recombinant and plasma PLTP on HDL subspecies. Biochim Biophys Acta 1995;1258:27–34.

[20] Jiang X-C, Bruce C. Regulation of murine plasma phospholipid transfer protein activity and mRNA levels by lipopolysaccharide and high cholesterol diet. J Biol Chem 1995;270:17133–8.

[21] Jiang X-C, Agellon LB, Walsh A, Breslow JL, Tall AR. Dietary cholesterol increases transcription of the human cholesteryl ester transfer protein gene in transgenic mice. Dependence on natural flanking sequences. J Clin Invest 1992;90:1290–5.