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Consumption of French-press coffee raises cholesteryl ester transfer protein activity levels before LDL cholesterol in normolipidaemic subjects

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Abstract. de Roos B, van Tol A, Urgert R, Scheek LM, van Gent T, Buytenhek R, Princen HMG, Katan MB (Wageningen University; Erasmus University, Rotterdam; and Gaubius Laboratory, Leiden; Wageningen Centre for Food Sciences, The Netherlands). Consumption of French-press coffee raises cholesteryl ester transfer protein activity levels before LDL cholesterol in normolipidaemic subjects. *J Intern Med* 2000; **248:** 211–216.

Objectives. To determine the long-term effects of unfiltered coffee consumption on the activity levels of cholesteryl ester transfer protein (CETP), phospholipid transfer protein (PLTP) and lecithin:cholesterol acyltransferase (LCAT) and to assess a possible role of CETP activity levels in the rise in serum LDL cholesterol.

Subjects and design. Forty-six healthy normolipidaemic subjects consumed 0.9 L of either French-press or filtered coffee for 24 weeks. Fasting blood samples were obtained after 0, 2, 12 and 24 weeks of intervention and after and 12 weeks of follow-up.

Main outcome measures. Serum activity levels of CETP, PLTP and LCAT.

Results. Relative to baseline, French-press coffee significantly increased average CETP activity by 12% after 2 weeks, by 18% after 12 weeks, and by 9% after 24 weeks. PLTP activity was significantly increased by 10% after 12 and 24 weeks. LCAT activity was significantly decreased by 6% after 12 weeks and by 7% after 24 weeks. The increase in CETP clearly preceded the increase in LDL cholesterol, but not the increase in total triglycerides. However, consumption of French-press coffee caused a persistent rise in CETP activity, whereas the rise in serum triglycerides was transient.

Conclusions. Consumption of cafestol and kahweol cause a long-term increase in CETP as well as PLTP activity; the increase in CETP activity may contribute to the rise in LDL cholesterol.

Keywords: cafestol, French-press coffee, kahweol, normolipidaemic subjects, serum lipid transfer proteins.

Introduction

Long-term consumption of unfiltered coffee potently raises serum low-density lipoprotein (LDL) cholesterol in humans [1]. The lipid-soluble diterpenes cafestol and kahweol – present in coffee beans [2] – are responsible for this effect [3]. Unfiltered coffee brews like French-press coffee, Scandinavian boiled coffee and Turkish coffee contain 3–6 mg of each diterpene per cup [2]. These diterpenes are the most potent cholesterol-raising substances from the diet that are known. Therefore, the action of cafestol and kahweol offers an interesting model to study the effect of dietary substances on cholesterol metabolism in humans.

The mechanism of action of cafestol and kahweol is poorly understood. *In vitro* studies show contradictory results in various cell lines. Cafestol decreased LDL receptor activity in human fibroblasts [4], in HepG2-cells [5] and in primary rat hepato-

cytes [6]. However, in CaCo2-cells, cafestol enhanced LDL cholesterol uptake and degradation [7]. We have previously found that, in humans, short-term consumption of diterpenes is associated with increased serum activity levels of the lipid transfer proteins cholesteryl ester transfer protein (CETP) and phospholipid transfer protein (PLTP) and with decreased activity levels of lecithin:cholesterol acyltransferase (LCAT) [8]. CETP catalyses the transfer of cholesteryl esters – synthesized by LCAT – from HDL to the apolipoprotein B-containing lipoproteins LDL and VLDL [9]. PLTP can affect the net mass transfer of phospholipids between lipoproteins [10] and it also converts small-sized HDL (HDL_3) into both larger (HDL_2-sized) and smaller (pre-β-migrating) HDL particles [11–13]. Both CETP and PLTP appear to play a major role in determining the concentration and size of HDL particles in plasma [10, 14]. In our earlier short-term study [8], it remained unclear whether the elevated serum CETP and PLTP activity levels in subjects consuming diterpenes were a cause or a consequence of the raised LDL cholesterol concentrations.

In the present study, we studied the long-term effects of French-press coffee consumption on serum lipid transfer proteins and LCAT. In addition, we compared the initial changes occurring in CETP activity levels with the initial changes in LDL and HDL cholesterol and total triglycerides during French-press coffee consumption. Filtered coffee was used as a control. We reasoned that an increase in CETP activity prior to an increase in LDL cholesterol could indicate a role of this protein in the mechanism of cafestol and kahweol to raise LDL cholesterol concentrations in humans.

Subjects and methods

Subjects

Approval for the experiments was obtained from the Human Ethics Committee of the Division of Human Nutrition & Epidemiology, Wageningen University. Forty-six healthy normolipidaemic subjects gave their written informed consent and participated in the study. The baseline characteristics of the subjects are indicated in Table 1. These subjects had a mean age of 30 years (range 19–69) and a body mass index (BMI) of 23 ± 1 (mean ± SEM) kg m^{-2}. Sixteen of these subjects were smokers. Alcohol consumption was limited to less than 3 beverages day^{-1} per subject.

Design

During a run-in period of 4 weeks, subjects consumed 0.9 L day^{-1} of filtered coffee. Subsequently, they were stratified by age then randomly allocated to consume 0.9 L day^{-1} of either filtered coffee (n = 24 subjects; 12 male and 12 female) or French-press coffee (n = 22 subjects; 11 male and 11 female) for 24 weeks. Subjects brewed their coffee at home. Filtered coffee was prepared by scooping 33 g of finely ground coffee into a paper filter and pouring 1.1 L of boiling water onto the grounds. Boiled coffee was prepared by scooping 39 g of coarse grounds into a cafetière (Kaffee Primo, BMF, Germany) and pouring 1.2 L of boiling water onto the grounds. Subjects stirred the brew for 10 s, allowed it to stand for 2–5 min, pushed down the plunger to separate the grounds from the brew, and then decanted the brew. This amount of French-press coffee provided about 38 mg day^{-1} of cafestol and 33 mg day^{-1} of kahweol. Filtered coffee provided less than 1 mg day^{-1} of either diterpene. Both coffee brews contained similar amounts of caffeine.

Blood sampling and assays

Fasting venous blood samples were taken after 0, 2, 12 and 24 weeks of intervention and after 12 weeks of follow-up. Serum was obtained by centrifugation and stored at −80°C. Total serum triglycerides, LDL cholesterol and HDL cholesterol were assayed as described [1]. Serum CETP activity levels were assayed in duplicate after removal of endogenous VLDL + LDL [15] using excess exogenous substrates. The isotope assay measures the transfer of [1-^{14}C-oleate]cholesteryl ester from labelled LDL to an excess of unlabelled pooled normal HDL, whilst LCAT is inhibited with dithiobis-2-nitrobenzoic acid [16]. CETP activity was calculated as the bidirectional transfer between labelled LDL and HDL. The CETP activity levels obtained by this method correlate well with CETP mass [17]. Serum PLTP activity levels were assayed in duplicate in a phospholipid vesicles-HDL system, as previously described [15]. In short, small serum samples of 0.5–1.0 μL were incubated with [^{3}H]phosphatidyl-

Table 1 Baseline characteristics of the subjects consuming filtered coffee (n = 24) or French-press coffee (n = 22) for 6 months. Values are means (± SEM) unless stated otherwise

	Filtered coffee	French-press coffee
Gender (n, male/female)	12/12	11/11
Age (years)	29 ± 2	30 ± 2
Body mass index (kg m^{-2})	22 ± 1	23 ± 1
Smoking (n, yes/no)[a]	10/14	6/16
Daily amount of alcoholic drinks[a]	1.0 ± 0.1	0.7 ± 0.2
Serum lipid levels		
Total cholesterol (mmol L^{-1})	5.0 ± 0.2	4.9 ± 0.2
LDL cholesterol (mmol L^{-1})[b]	3.0 ± 0.2	3.0 ± 0.2
Triglycerides (mmol L^{-1})	1.1 ± 0.1	1.1 ± 0.1
Serum lipid transfer proteins		
Cholesteryl ester transfer protein (%)	105.0 ± 5.4	107.7 ± 6.2
Phospholipid transfer protein (%)	95.5 ± 2.9	91.6 ± 3.4
Lecithin:cholesterol acyltransferase (%)	95.5 ± 2.2	95.7 ± 2.6

[a]Self-reported consumption.
[b]Calculated according to Friedewald *et al.* [36].

choline-labelled liposomes and an excess of pooled normal HDL, followed by precipitation of the liposomes with a mixture of NaCl, $MgCl_2$ and heparin (final concentrations of 230 mmol L^{-1}, 32 mmol L^{-1} and 200 U mL^{-1}, respectively). The measured PLTP activity is not influenced by the phospholipid transfer promoting properties of CETP [15]. Serum LCAT activity levels were measured in duplicate, using excess exogenous substrate containing [^{3}H]cholesterol as described [18]. The measured activity is indicative for the serum LCAT concentration [19]. Serum CETP, PLTP and LCAT activity levels were related to human pool serum and expressed in arbitrary units (AU, which corresponds to the percentages of the activities present in the serum pool). All subjects were analysed using one batch of substrates. The within-assay coefficients of variation of CETP, PLTP and LCAT were 2.7, 3.5 and 4.5%, respectively.

Statistics

Responses were calculated by subtracting baseline values from values obtained during treatment. All variables were normally distributed. Differences in responses between the French-press and filtered-coffee group were tested against zero with unpaired t-tests.

Results

Consumption of French-press coffee significantly increased CETP activity by 12% after 2 weeks, by 18% after 12 weeks and by 9% after 24 weeks. PLTP activity was significantly increased by 10% after 12 and 24 weeks. LCAT activity was significantly decreased by 6% after 12 weeks and by 7% after 24 weeks. Both CETP and PLTP activity dropped just below baseline after consumption of French-press coffee had ceased. LCAT returned to baseline after cessation of treatment (Fig. 1).

After 2 weeks of French-press coffee consumption, CETP had already reached 68% of the maximal increase that was reached after 12 weeks of intervention. The increase in LDL cholesterol only became significant after 12 weeks. At 2 weeks, LDL cholesterol had only reached 18% of the maximal increase that was reached after 12 weeks of intervention. As reported previously [1], triglycerides, and thus presumably VLDL, had already peaked at the first measuring point, after which levels slowly returned to baseline. No significant changes were detected in HDL cholesterol (Fig. 1).

Discussion

Long-term consumption of French-press coffee increased CETP and PLTP activity and decreased LCAT activity in a group of healthy normolipidaemic

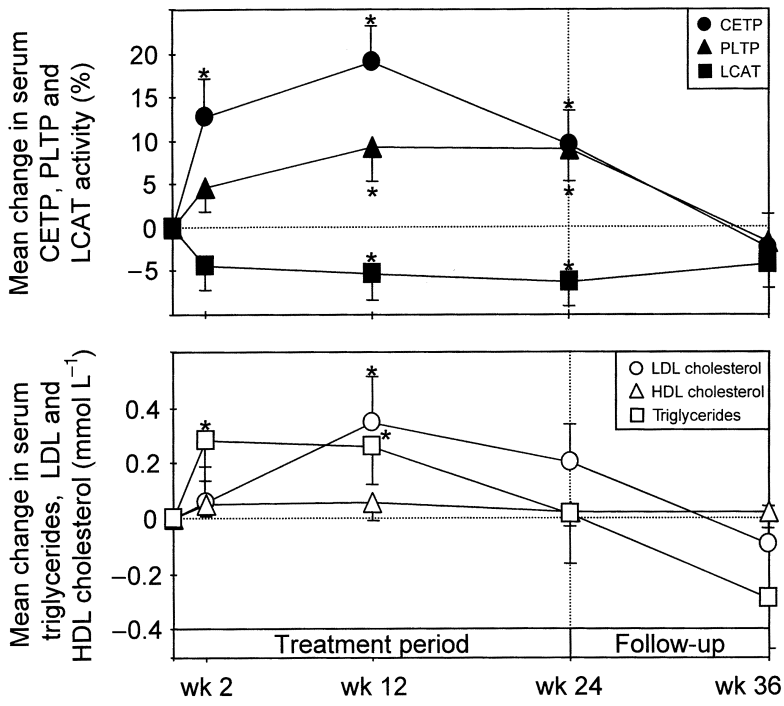

Fig. 1 Mean changes from baseline values in serum activity levels of CETP, PLTP and LCAT and in serum concentrations of LDL and HDL cholesterol and serum triglycerides in 22 subjects drinking 0.9 L day^{-1} of French-press coffee for 24 weeks. For each time point, mean changes from baseline in 24 subjects drinking filtered coffee were subtracted from those in the subjects drinking French-press coffee. The error bars represent the standard error of the mean difference. Statistical differences between the two intervention groups ($P < 0.05$) are indicated by an asterisk (*).

subjects. We also found that consumption of French-press coffee increased CETP activity before it increased LDL cholesterol. During consumption of French-press coffee, CETP activity levels had already reached 68% of their maximum increase at the first measurement point, 2 weeks after subjects had started to drink unfiltered coffee rich in cafestol. At that point in time, LDL levels had reached only 18% of their maximum increase (Fig. 1).

The present findings extend our earlier short-term data obtained with purified coffee diterpenes [8]. In our previous experiments, which lacked frequent sampling at early time periods, it remained unclear whether elevated activity levels of CETP and PLTP due to cafestol or kahweol consumption were a cause or a consequence of elevated serum LDL cholesterol levels [8]. Earlier human studies on the effects of a high-fat, high-cholesterol diet on serum CETP also do not provide clear evidence for a causal relationship, e.g. in favour of a mechanism with increased serum CETP activity levels causing increased serum LDL cholesterol levels [20–22]. Data from animal models are not conclusive either. Some studies in transgenic mice suggest that increased CETP levels may be secondary to increased LDL cholesterol levels. When mice carrying the human CETP gene were cross-bred with hyperlipidaemic mice which had a deficiency in either apolipoprotein E or the LDL receptor, plasma CETP levels were increased to values higher than those found in normolipidaemic mice carrying the human CETP gene [23]. However, other studies showed that expression of the monkey CETP gene in mice raised plasma levels of LDL, a class of lipoproteins which is virtually absent in wild-type mice [24]. Rats injected intravenously with purified human CETP also showed increased LDL and apolipoprotein B concentrations [25]. In the present study, the increase in CETP activity levels preceded the increase in LDL cholesterol, although triglycerides, and thus presumably VLDL, rose rapidly on cafestol. One could argue that the rise in CETP may itself have been caused by the rise in VLDL. However, serum triglycerides levels returned to baseline after 2 weeks of French-press coffee consumption, whereas the CETP activity remained significantly increased (Fig. 1). This finding might therefore support a role for CETP in the effect of cafestol on LDL levels in humans.

PLTP activity also increased upon long-term French-press coffee consumption. This is in agreement with data from our previous study in which subjects consumed coffee diterpenes [8]. PLTP and CETP belong to the same lipid transfer protein/

lipopolysaccharide binding protein (LTP/LBP) gene family [26]. Both proteins are upregulated by cholesterol feeding in animal studies [27, 28], suggesting that their genes contain cholesterol-responsive elements [9]. A parallel increase in both lipid transfer proteins might therefore be expected. Serum LCAT activity levels decreased slightly after consumption of French-press coffee. A comparable decrease was observed earlier after consumption of purified coffee diterpenes [8]. Since LCAT is solely synthesized in the liver [19], an impairment of liver function, as evident from increased activities of serum transaminases after French-press coffee consumption [1], may explain the decrease in serum LCAT levels. Because LCAT primarily functions in the formation of HDL cholesteryl esters, a lower LCAT activity may result in decreased HDL cholesterol levels, especially when CETP activity is increased. Indeed, cafestol and kahweol were found to lower serum HDL cholesterol in one previous study [3], although not in other studies [29, 30], nor in the present study. However, HDL cholesteryl esters can have two different metabolic fates: CETP-mediated transfer to triglyceride-rich lipoproteins [9], or direct delivery to (liver) cells via the scavenger receptor class B type I (SR-BI) [31]. The mechanisms involved in the control of SR-BI are still unclear. The promoter region of the human SR-BI gene contains consensus-DNA binding sequences for several transcription factors, including sterol regulatory element binding proteins (SREBP). Transcriptional regulation by SREBP or other factors is the most likely means of regulation of SR-BI expression by dietary cholesterol and fatty acids *in vivo* [32]. In analogy, cafestol might affect the expression of SR-BI, thereby influencing levels of HDL cholesterol in the circulation.

If CETP is involved in the mechanism of action of coffee diterpenes then it might increase the cholesteryl ester content of VLDL and/or LDL [33]. The effect of coffee diterpenes on the composition of the various lipoproteins is not known. Two earlier studies showed a significant increase in apolipoprotein B100 levels of 0.09 [34] and 0.13 g L^{-1} [35] after consumption of boiled coffee. This suggests that the increase in LDL cholesterol is at least partly explained by changes in the number of LDL particles. An additional change in lipoprotein composition cannot, however, be excluded. Since the rise in CETP activity preceded the rise in LDL cholesterol upon consumption of French-press coffee, CETP might contribute to the LDL cholesterol-raising effect of coffee diterpenes.

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