

Coffee Oil Consumption Increases Plasma Levels of 7 α -Hydroxy-4-cholesten-3-one in Humans¹

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ABSTRACT Unfiltered coffee brews such as French press and espresso contain a lipid from coffee beans named cafestol that raises serum cholesterol in humans. Cafestol decreases the expression and activity of cholesterol 7 α -hydroxylase, the rate-limiting enzyme in the classical pathway of bile acid synthesis, in cultured rat hepatocytes and livers of APOE3Leiden mice. Inhibition of bile acid synthesis has been suggested to be responsible for the cholesterol-raising effect of cafestol. Therefore, we assessed whether cafestol decreases the activity of cholesterol 7 α -hydroxylase in humans. Because liver biopsies were not feasible, we measured plasma levels of 7 α -hydroxy-4-cholesten-3-one, a marker for the activity of cholesterol 7 α -hydroxylase in the liver. Plasma 7 α -hydroxy-4-cholesten-3-one was measured in 2 separate periods in which healthy volunteers consumed coffee oil containing cafestol (69 mg/d) for 5 wk. Plasma levels of 7 α -hydroxy-4-cholesten-3-one increased by 47 \pm 13% (mean \pm SEM, n = 38, P = 0.001) in the first period and by 23 \pm 10% (n = 31, P = 0.03) in the second treatment period. Serum cholesterol was raised by 23 \pm 2% (P < 0.001) in the first period and by 18 \pm 2% (P < 0.001) in the second period. We corrected individual 7 α -hydroxy-4-cholesten-3-one levels for serum cholesterol levels, because coffee oil increases serum cholesterol and 7 α -hydroxy-4-cholesten-3-one is probably present in the lipoprotein fraction of serum. After correction, the increase in 7 α -hydroxy-4-cholesten-3-one was 24 \pm 11% (P = 0.04) in the first period and there was no effect in period 2. Our study showed that coffee oil did not decrease, and actually increased, plasma levels of 7 α -hydroxy-4-cholesten-3-one in humans in 2 separate treatment periods. Therefore, this study does not support the hypothesis that cafestol decreases bile acid synthesis in humans. *J. Nutr.* 135: 785–789, 2005.

KEY WORDS: • coffee oil • cafestol • cholesterol • bile acid synthesis

Humans and animals regulate their metabolism in response to dietary compounds. Elucidation of underlying molecular mechanisms using in vitro and animal studies allows us to identify novel dietary components that can affect metabolism and potentially health. However, before one can apply knowledge of molecular mechanisms to identify such compounds in the human diet, it must be established that the potential mechanism indeed is important in humans.

In this study we used coffee oil to explore a new potential pathway for the mode of action of food components that affect serum lipids. Oil from coffee beans contains the diterpenes cafestol and kahweol, which are responsible for the cholesterol-raising effect of unfiltered coffee types (1–4). Cafestol raises serum cholesterol more potently than the related diterpene kahweol, which is also found in coffee beans (3). The combined results of 11 intervention trials show that each 10 mg of cafestol after 4 wk of daily consumption raises serum total

cholesterol by 0.13 mmol/L (4). Therefore, elucidation of the mechanism by which cafestol achieves this large effect on serum cholesterol would be helpful in understanding how certain food components can affect lipid metabolism. A number of potential mechanisms have been proposed, such as downregulation of the LDL receptor (5) and regulation of lipid transfer proteins such as cholesteryl ester transfer protein, phospholipids transfer protein, and lecithin:cholesterol acyltransferase (6,7). However, it remains unclear by which mechanism cafestol affects these proteins.

Cafestol feeding causes a rise in serum cholesterol in APOE3Leiden mice after 3 wk. This rise is accompanied by a decrease in cholesterol 7 α -hydroxylase (Cyp7a1)³ expression of 58% (8) and fecal bile acid content is reduced by 41% after cafestol feeding (8). Cyp7a1 is the rate-limiting enzyme in the classical pathway of bile acid synthesis from cholesterol in the liver. When rats or mice are fed cholesterol, Cyp7a1 is up-regulated; this enzyme converts the extra cholesterol into bile

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³ Abbreviations used: ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; Cyp7a1, cholesterol 7 α -hydroxylase; LXR, liver X receptor; PXR, pregnane X receptor.

acids, which are excreted with the feces. In this way, mice do not exhibit the rise in serum cholesterol induced by dietary cholesterol that occurs in other species.

Suppression of bile acid synthesis increases the levels of hepatic cholesterol and this could lead to downregulation of the LDL receptor. This could explain the increase in serum LDL-cholesterol in APOE3Leiden mice fed cafestol. In humans, cafestol intake also increases serum LDL-cholesterol. Whether this is caused by a decrease in Cyp7a1 activity is not known. However, Cyp7a1 deficiency in humans does increase serum lipid levels (9). Due to the differences between mice and humans in regulation of bile acid synthesis, it is essential to establish whether cafestol also regulates activity of Cyp7a1 in humans.

Because it is ethically unacceptable to take liver biopsies to study the effect of cafestol on expression and activity of Cyp7a1 in human livers, we used an indirect method. In this method, the level of 7 α -hydroxy-3-cholesten-4-one, a metabolite of cholesterol, is measured in plasma. 7 α -Hydroxy-3-cholesten-4-one is an intermediate in bile acid synthesis and its level in human plasma is considered to reflect Cyp7a1 activity in the liver (10–12).

METHODS

Subjects. Subjects were recruited and screened as previously described (13). Fifty subjects, including 13 men and 37 women, were enrolled. During the study 5 subjects withdrew: 3 subjects suffered from stomach complaints, 1 went abroad, and 1 had a gastrointestinal infection. Another 13 subjects were excluded during the study because their serum activities of alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) exceeded previously determined boundaries of 2.7 times the upper limit of normal for ALAT and 1.5 times the upper limit of normal for ASAT. The Medical Ethical Committee of Wageningen University and Research Centre approved the study. Each volunteer gave written informed consent.

Study design. The study was originally designed to assess the within-subject reproducibility of the serum lipid response to coffee oil. Therefore, the response to coffee oil was measured in 2 separate treatment periods (13).

Subjects first entered a run-in of 3 wk in which they received 4 placebo capsules daily. Placebo capsules each contained 0.25 mL sunflower oil and 0.25 mL safflower oil to mimic the fatty acid composition of coffee oil (14). After the run-in subjects took 4 coffee-oil capsules a day (2 mL/d) for 5 wk. The coffee oil capsules provided 69 mg cafestol and 51 mg of kahweol per day. This dose is similar to the amount present in 10 cups (150 mL) of boiled coffee (4). The run-in and the coffee oil stage together constituted period 1. The change in the level of serum lipids from the end of run-in 1 to the end of coffee oil stage 1 was defined as response 1. This first coffee oil stage was followed by a 3-wk wash-out period during which no capsules were supplied. After the wash-out period, subjects repeated the first 2 stages: a 3-wk run-in (run-in 2) and a 5-wk coffee oil stage (coffee oil stage 2) (Fig. 1).

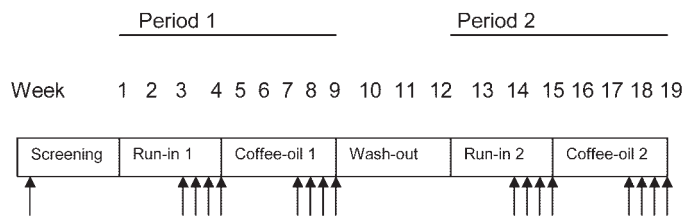


FIGURE 1 Diagram of the study design. Subjects received placebo capsules during the run-in stage and coffee oil capsules during the coffee oil stage in each of 2 separate periods. The week number is indicated. Black arrows indicate blood sampling days.

Subjects were asked to maintain their lifestyles and dietary habits for the duration of the study. They reported in diaries changes in diet, smoking, physical activity, use of medication, illness, and the number of capsules taken daily.

Laboratory measurements. Blood samples were taken after subjects had fasted overnight on 4 separate days in the last 2 wk of the run-in and coffee oil stages. Both serum and plasma samples were prepared. Levels of total cholesterol, HDL-cholesterol, triglycerides, ALAT, and ASAT were measured in the 16 serum samples of each person as previously described (13). LDL-cholesterol levels were calculated (15).

In addition, a number of liver function markers were measured in the serum samples: alkaline phosphatase, bilirubin, γ -glutamyl transpeptidase, and lactate dehydrogenase. We also measured levels of amylase, a marker for pancreatitis. All tests were performed using Flex reagents cartridges with the Dimension system (Dade Behring). Levels of bile acids were also determined in serum using an Enzabile kit (Bio-Stat Diagnostic Systems).

For the analysis of the bile acid precursor 7 α -hydroxy-4-cholesten-3-one we pooled plasma samples per subject. This yielded 4 plasma samples, 1 for each run-in and 1 for each coffee oil stage. We determined plasma levels of 7 α -hydroxy-4-cholesten-3-one using the HPLC method described by Gälman et al. (12). We were not able to measure 7 α -hydroxy-4-cholesten-3-one levels in samples of 2 subjects in period 1 and of 1 subject in period 2 due to the presence of an unidentified compound in the plasma that interfered with our internal standard. In total we measured 7 α -hydroxy-4-cholesten-3-one levels in samples of 38 subjects in period 1 and of 31 subjects in period 2. The 4 samples were analyzed within 1 run. The within-run CV was 9.7%.

Statistics. A subject's response to coffee oil in period 1 was defined as the mean level at the end of coffee oil stage 1 minus the mean at the end of run-in 1. For the samples in which liver function variables and bile acids were determined, the mean for a period was calculated as the mean of the 4 repeated measurements in each stage. The response in period 2 was calculated in the same way. We compared means using Student's *t* test for paired samples. Values are means \pm SEM. Differences were considered significant at $P < 0.05$.

RESULTS

Changes in variables during each of the 2 treatment periods are presented in **Table 1**. In the text of this section, the results are presented as the change over the 2 periods combined. All changes presented were significant ($P < 0.05$).

Serum lipids. Responses of serum lipids to coffee oil in this study were previously published (13). The concentrations presented here are the recalculated values after omission of the serum lipid responses of subjects for which no data on 7 α -hydroxy-4-cholesten-3-one were available. Serum total cholesterol was raised by 21%, triglycerides were raised by 60%, and LDL-cholesterol was raised by 24%. HDL-cholesterol levels were not affected by coffee oil treatment.

Liver function and bile acids. ALAT levels were raised by 112% after coffee oil treatment and ASAT levels were raised by 46%. Serum alkaline phosphatase was decreased by 10% and γ -glutamyltranspeptidase by 15%. Serum amylase was not changed in period 1 and was decreased by 5% in period 2. The coffee oil treatment did not affect serum bilirubin or lactate dehydrogenase.

7 α -Hydroxy-4-cholesten-3-one and bile acid response to coffee oil. Plasma 7 α -hydroxy-4-cholesten-3-one increased over both periods by 35%. There was no correlation ($n = 30$, $r = -0.02$) between the individual 7 α -hydroxy-4-cholesten-3-one responses in the 2 treatment periods. Plasma oxysterols are mostly found in the lipoprotein fractions (16). Therefore, a rise in the plasma level of 7 α -hydroxy-4-cholesten-3-one could be explained by an increase in levels of lipoprotein fractions in plasma, analogous to serum vitamin E levels that

TABLE 1

Effect of coffee-oil consumption on plasma 7 α -hydroxy-4-cholesten-3-one, serum lipids, and liver function variables in humans during 2 separate study periods¹

Variable	Run-in 1 ²	Response period 1 ³	Run-in 2	Response period 2
	<i>n</i> = 38	<i>n</i> = 38	<i>n</i> = 31	<i>n</i> = 31
7 α -Hydroxy-4-cholesten-3-one, $\mu\text{g/L}$	7.5 \pm 1.0	3.5 \pm 1.0**	8.1 \pm 0.7	1.8 \pm 0.8*
Total cholesterol, <i>mmol/L</i>	4.4 \pm 0.1	1.0 \pm 0.1**	4.4 \pm 0.1	0.8 \pm 0.1**
HDL, <i>mmol/L</i>	1.5 \pm 0.1	0 \pm 0	1.5 \pm 0.1	0 \pm 0
LDL, <i>mmol/L</i>	2.4 \pm 0.1	0.7 \pm 0.1**	2.4 \pm 0.1	0.5 \pm 0.1**
Triglycerides, <i>mmol/L</i>	1.1 \pm 0.1	0.7 \pm 0.1**	1.1 \pm 0.1	0.7 \pm 0.1**
Alanine aminotransferase, <i>IU/L</i>	17 \pm 1	27 \pm 5**	20 \pm 2	14 \pm 2**
Aspartate aminotransferase, <i>IU/L</i>	17 \pm 1	12 \pm 3**	18 \pm 1	5 \pm 1**
Alkaline phosphatase, <i>U/L</i>	64 \pm 3	-5 \pm 1**	67 \pm 3	-9 \pm 1**
Amylase, <i>U/L</i>	75 \pm 4	3 \pm 2	73 \pm 5	-4 \pm 1*
Bile acids, $\mu\text{mol/L}$	3.3 \pm 0.3	0.2 \pm 0.3	2.9 \pm 0.3	0.4 \pm 0.3
Bilirubin, $\mu\text{mol/L}$	12.6 \pm 0.7	-0.9 \pm 0.5	12.7 \pm 0.6	0.4 \pm 0.5
γ -Glutamyltranspeptidase, <i>U/L</i>	14.9 \pm 1.0	-0.7 \pm 0.6	18.0 \pm 1.6	-4.6 \pm 0.6**
Lactate dehydrogenase, <i>U/L</i>	276 \pm 7	1 \pm 6	273 \pm 7	-6 \pm 4

¹ Values are means \pm SEM. Asterisks indicate different from the corresponding run-in: * P < 0.05, ** P < 0.01.

² Three-week run-in stage in which placebo capsules were consumed.

³ Responses are the increase or decrease from the run-in levels after 5 wk of coffee oil treatment.

are also customarily corrected for lipoprotein concentrations. Cafestol treatment increased cholesterol in serum (Table 1). To correct for this increase in serum cholesterol, we divided the individual levels of 7 α -hydroxy-4-cholesten-3-one by the corresponding cholesterol concentration. The increase in 7 α -hydroxy-4-cholesten-3-one after correction was 24 \pm 11% in period 1 and there was no effect in period 2 (Fig. 2). Cafestol did not affect serum bile acid concentrations.

DISCUSSION

In this study we showed that cafestol increased plasma 7 α -hydroxy-4-cholesten-3-one levels during 2 separate treatment periods in humans. This suggests that Cyp7a1 activity in the liver was not decreased by the coffee oil treatment. Previous studies observed a decrease in expression and activity of Cyp7a1 after treatment with cafestol in cultured rat hepatocytes and livers of APOE3Leiden mice (17). Based on these studies, we expected a decrease in Cyp7a1 activity and therefore in plasma 7 α -hydroxy-4-cholesten-3-one in humans upon cafestol treatment. We calculated that this study would enable us to detect a decrease or increase in 7 α -hydroxy-4-cholesten-3-one levels of at least 20% (2-sided α = 0.05 and power 1 - β = 80%). The other variables measured in the serum of subjects in this study showed the typical response to cafestol: total cholesterol and LDL-cholesterol increased as did the liver enzymes, ALAT and ASAT (1,4). Furthermore, alkaline phosphatase decreased during both periods and γ -glutamyltranspeptidase decreased during treatment period 2, as has been observed in previous studies (1,4,18).

The increases in 7 α -hydroxy-4-cholesten-3-one levels can be partly explained by the increase in serum cholesterol. Due to its lipophilic nature, 7 α -hydroxy-4-cholesten-3-one is, like other oxysterols, probably present in the lipoprotein fractions. However, this does not explain why we did not observe a decrease in 7 α -hydroxy-4-cholesten-3-one levels as we expected. Due to the consecutive design of the study we cannot exclude the possibility of a seasonal effect on plasma levels of 7 α -hydroxy-4-cholesten-3-one. However, after the wash-out between periods 1 and 2, the plasma level of 7 α -hydroxy-4-cholesten-3-one returned to the level at the beginning of period 1 and therefore, it is unlikely that the observed increase

was due to seasonal variation. We measured 7 α -hydroxy-4-cholesten-3-one levels in the last 2 wk of both coffee oil treatment stages. It is possible that Cyp7a1 activity, and therefore 7 α -hydroxy-4-cholesten-3-one levels, were decreased in the first 3 wk of the treatment stages. However, it would then be unlikely that the effect of cafestol on Cyp7a1 contributes to

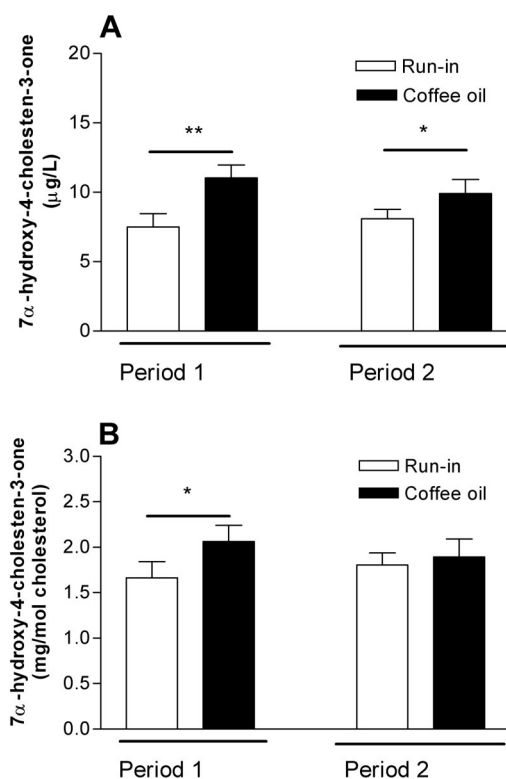


FIGURE 2 Plasma levels of 7 α -hydroxy-3-cholesten-4-one (A) and 7 α -hydroxy-3-cholesten-4-one corrected for serum cholesterol levels (B) before and after coffee oil treatment in humans after 2 periods of consumption. Values are means \pm SEM, *n* = 38 (period 1) or 31 (period 2). Asterisks indicate a difference from the run-in period, * P < 0.05, ** P < 0.01.

the serum lipid response, because the effect on LDL-cholesterol is still present after 6 months of daily consumption of French press coffee (18). Furthermore, the influence of diurnal variation on the outcome of this study is also unlikely, because blood was taken at the same time each blood-sampling day.

The levels of 7 α -hydroxy-4-cholesten-3-one we measured in pooled plasma reflect the concentration on 4 different blood sampling days spread over 2 wk. Therefore, it is possible that day-to-day variation affected the response measured. However, the serum lipid response to cafestol is already present and persistent in wk 4 and 5 of cafestol treatment (18).

It is possible that the response of 7 α -hydroxy-4-cholesten-3-one to cafestol in plasma of humans does not reflect the change in Cyp7a1 activity in liver. However, in patients with diseases that are associated with a change in bile acid production, plasma levels of 7 α -hydroxy-4-cholesten-3-one reflect Cyp7a1 activity in the liver (10,11). Furthermore, serum levels of 7 α -hydroxy-4-cholesten-3-one are closely correlated with the synthesis rate of chenodeoxycholic acid and cholic acid in humans (19). To our knowledge, it is not known whether the level of 7 α -hydroxy-4-cholesten-3-one reflects the response of Cyp7a1 activity to dietary changes in humans. However, treatment of patients with chenodeoxycholic acid reduced serum levels of 7 α -hydroxy-4-cholesten-3-one by >80% in 2 patients (20). This suggests that this marker responds to treatment. In addition, serum levels of 7 α -hydroxy-4-cholesten-3-one follow diurnal variation in Cyp7a1 activity in rat liver (12). In rabbits, cholesterol feeding increases Cyp7a1 activity, while bile drainage decreases it. The response of 7 α -hydroxy-4-cholesten-3-one in serum is correlated with Cyp7a1 activity in rabbit livers after correction for the response of serum cholesterol (21). Together, these studies indicate that serum levels of 7 α -hydroxy-4-cholesten-3-one reflect the activity of Cyp7a1 in the liver.

Another possibility is that 7 α -hydroxy-4-cholesten-3-one in the plasma does not reflect a change in Cyp7a1 activity upon treatment with cafestol specifically. Interestingly, a study in which subjects were treated with the antibiotic rifampicin showed a 70% increase in serum levels of 7 α -hydroxy-4-cholesten-3-one (22). This study also showed that levels of deoxycholic acid decreased upon treatment with rifampicin, suggesting that rifampicin upregulates clearance of these secondary bile acids, which can result in upregulation of Cyp7a1 activity via an FXR-mediated negative-feedback mechanism. However, we did not observe a decrease in serum levels of bile acids upon coffee oil treatment. Moreover, in feces of APOE3Leiden mice that were fed cafestol, total bile acid content was reduced by 41%, while the relative content of primary and secondary bile acids was not affected. Therefore, it is unlikely that cafestol increases the clearance of secondary bile acids and subsequently upregulates Cyp7a1 activity. Like rifampicin, cafestol regulates the activity of several enzymes involved in detoxification (23). It is possible that cafestol disturbs the clearance of 7 α -hydroxy-4-cholesten-3-one itself. If this is indeed the case, 7 α -hydroxy-4-cholesten-3-one is not a useful marker for the change in activity of Cyp7a1 upon treatment with such compounds. However, it remains to be established whether cafestol indeed disturbs clearance of 7 α -hydroxy-4-cholesten-3-one.

Detrimental effects of cafestol on hepatocytes could also be involved in the response of 7 α -hydroxy-4-cholesten-3-one to cafestol in plasma. Previous studies showed that cafestol increases the liver enzymes ALAT and ASAT (1,4). This suggests that injury to hepatocytes occurs. However, it is not clear whether injury to hepatocytes would cause an increase of 7 α -hydroxy-4-cholesten-3-one in plasma.

Previous studies showed that several animals including monkeys, hamsters, rabbits, wild-type rats, and gerbils do not show an increase in LDL-cholesterol upon cafestol feeding (24). There is a precedent for differences in regulation of bile acid metabolism between mice and humans, and that is the regulation of the Cyp7a1 gene by the liver X receptor (LXR). In mice, oxysterols activate LXR and this upregulates Cyp7a1 expression and bile acid synthesis (25–27). In humans, activation of LXR has no effect on Cyp7a1 expression and bile acid synthesis. This is explained by the absence of an LXR response element in the human Cyp7a1 gene (27,28). Another example is the fact that the human and mouse orthologues of the pregnane X receptor (PXR) are activated by different ligands (29). Moreover, the genes that are regulated by PXR differ between species due to differences between the receptor orthologues (30).

We conclude that coffee oil containing cafestol did not decrease and, in fact, increased plasma levels of 7 α -hydroxy-4-cholesten-3-one in humans. This suggests that Cyp7a1 activity and bile acid synthesis are not decreased. Therefore, this study does not support the hypothesis that cafestol raises serum cholesterol by decreasing the synthesis of bile acids. Furthermore, elucidation of the mechanism behind the cholesterol-raising effect of cafestol is hampered by differences between species in the regulation of bile acid and cholesterol metabolism. Animal models are widely used for studying lipid metabolism, but caution is necessary when translating results from animal studies to human situations. Mechanisms elucidated in animals often remain to be tested in humans.

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