

Cafestol Increases Serum Cholesterol Levels in Apolipoprotein E*3-Leiden Transgenic Mice by Suppression of Bile Acid Synthesis

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Abstract—Cafestol, a diterpene present in unfiltered coffee, potently increases serum cholesterol levels in humans. So far, no suitable animal model has been found to study the biochemical background of this effect. We determined the effect of cafestol on serum cholesterol and triglycerides in different mouse strains and subsequently studied its mechanism of action in apolipoprotein (apo) E*3-Leiden transgenic mice. ApoE*3-Leiden, heterozygous low density lipoprotein–receptor (LDLR+/-) knockout, or wild-type (WT) C57BL/6 mice were fed a high- (0.05% wt/wt) or a low- (0.01% wt/wt) cafestol diet or a placebo diet for 8 weeks. Standardized to energy intake, these amounts are equal to 40, 8, or 0 cups of unfiltered coffee per 10 MJ per day in humans. In apoE*3-Leiden mice, serum cholesterol was statistically significantly increased by 33% on the low- and by 61% on the high-cafestol diet. In LDLR+/- and WT mice, the increases were 20% and 24%, respectively, on the low-cafestol diet and 55% and 46%, respectively, on the high-cafestol diet. These increases were mainly due to a rise in very low density lipoprotein (VLDL) and intermediate density lipoprotein cholesterol in all 3 mouse strains. To investigate the mechanism of this effect, apoE*3-Leiden mice were fed a high-cafestol or a placebo diet for 3 weeks. Cafestol suppressed enzyme activity and mRNA levels of cholesterol 7 α -hydroxylase by 57% and 58%, respectively. mRNA levels of enzymes involved in the alternate pathway of bile acid synthesis, ie, sterol 27-hydroxylase and oxysterol 7 α -hydroxylase, were reduced by 32% and 48%, respectively. The total fecal bile acid output was decreased by 41%. Cafestol did not affect hepatic free and esterified cholesterol, but it decreased LDLR mRNA levels by 37%. The VLDL apoB and triglyceride production rates, as measured after Triton injection, were 2-fold decreased by cafestol, indicating that the number of particles secreted had declined and that there was no change in the amount of triglycerides present in the VLDL particle during cafestol treatment. However, the VLDL particles contained a 4-times higher amount of cholesteryl esters, resulting in a net 2-fold increased secretion of cholesteryl esters. The decrease in triglyceride production was the result of a reduction in hepatic triglyceride content by 52%. In conclusion, cafestol increases serum cholesterol levels in apoE*3-Leiden mice by suppression of the major regulatory enzymes in the bile acid synthesis pathways, leading to decreased LDLR mRNA levels and increased secretion of hepatic cholesterol esters. We suggest that suppression of bile acid synthesis may provide an explanation for the cholesterol-raising effect of cafestol in humans. (*Arterioscler Thromb Vasc Biol.* 2000;20:1551-1556.)

Key Words: bile acid synthesis ■ cholesterol 7 α -hydroxylase ■ sterol 27-hydroxylase
■ apolipoprotein E*3-Leiden mice ■ cafestol

Unfiltered coffee markedly increases serum cholesterol levels in humans. The compounds responsible for this effect are cafestol and kahweol, 2 diterpenes that are present in coffee beans.¹ From our experiments, cafestol appeared to be by far the more potent compound.² We estimated that each 10 mg of cafestol ingested per day raises serum cholesterol levels by 0.13 mmol/L.³ In humans, \approx 80% of the rise in total cholesterol is accounted for by LDL cholesterol, and the rest is due to a rise in VLDL cholesterol.⁴

The mechanism by which coffee diterpenes influence lipid metabolism is largely unknown. Recently, we reported that cafestol suppressed bile acid synthesis in cultured rat hepatocytes by downregulation of cholesterol 7 α -hydroxylase and sterol 27-hydroxylase.⁵ Suppression of bile acid synthesis will lead to an increased pool of regulatory cholesterol, resulting in decreased expression of the hepatic LDL receptor (LDLR). This may provide an explanation for the cholesterol-raising effect of cafestol in humans.⁵ The availability of an

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animal model to study this hypothesis in vivo would be of great value, since it may help us to validate our in vitro experiments and eventually to discover the metabolic control points of cafestol. However, in previous studies, various animal models like hamsters⁶⁻⁸; rats^{6,9}; gerbils⁸; and Cebus, Rhesus, and African green monkeys¹⁰ did not respond to cafestol and kahweol as humans do, regardless of the dosage, the mode of administration, or the duration of treatment.

Therefore, we studied the effects of cafestol and kahweol on serum lipoproteins in apolipoprotein E*3-Leiden (apoE*3-Leiden) transgenic mice, in heterozygous LDLR-deficient (LDLR+/-) mice, and in wild-type (WT) C57BL/6 mice. We chose to use transgenic mice that overexpress human apoE*3-Leiden because these mice are highly susceptible to diet-induced hyperlipoproteinemia primarily due to a partial defect in the hepatic uptake of remnant lipoproteins.^{11,12} Because it was anticipated from our studies in cultured rat hepatocytes that cafestol would have an indirect effect on the expression of the LDLR,⁵ experiments were also performed with LDLR+/- mice. Lipoprotein profiles from LDLR+/- mice are more similar to those in humans than are those from WT mice.¹³ The cholesterol-raising effect of cafestol appeared to be most pronounced in apoE*3-Leiden mice, allowing us to investigate the mechanism of the cholesterol-raising effect of cafestol in these mice.

Methods

Animals, Housing, and Diets

Twenty-four female apoE*3-Leiden, 24 female LDLR+/-, and 24 female WT C57BL/6 mice were kept on a 12-hour dark/light cycle and allowed free access to food and water. Mice were fed a common challenge diet enriched with saturated fat and cholesterol (18.2 MJ/kg) containing (per 100 g) the following ingredients: cacao butter 15 g, corn oil 1 g, cholesterol 0.25 g, sucrose 40.5 g, corn starch 10 g, cellulose 5.9 g, casein 20 g, minerals 5.1 g, choline chloride 1 g, and methionine 0.2 g (Hope Farms). This diet was supplemented with 0.05% (wt/wt) cafestol and 0.025% (wt/wt) kahweol (high-cafestol diet), 0.01% (wt/wt) cafestol and 0.005% (wt/wt) kahweol (low-cafestol diet), or placebo. Standardized to daily energy intake, these amounts are comparable to a daily consumption of 40, 8, or 0 cups of unfiltered coffee, respectively, per 10 MJ (the average daily energy intake in humans). Institutional guidelines for animal care were observed in all experiments.

Experimental Design

Per mouse strain, animals were randomly divided into 3 experimental groups of 8 mice each and were matched by age. During a run-in period of 4 weeks, all mice received the placebo diet. During the treatment period, the groups consumed either the high- or low-cafestol diet or the placebo diet. Blood was collected at weeks 0, 2, 4, and 8 of the treatment period after an overnight fasting period. After 8 weeks of treatment, mice were bled and killed.

Measurement of Serum Lipids and Lipoproteins

In serum, total cholesterol and triglycerides were measured enzymatically (CHOD-PAP method, Boehringer Mannheim No. 236691, and GPO-trinder, Sigma No. 337-B, respectively). Alanine aminotransferase was measured enzymatically (GPT, Boehringer Mannheim No. 745138) in pooled sera from 4 mice. Serum lipoproteins were separated by ultracentrifugation. Two hundred microliters of pooled sera per group was layered with 1 mL of KBr ($\rho=1.21$), 2.58 mL of NaCl ($\rho=1.063$), and 8 mL of distilled water and centrifuged for 18 hours at 40 000 rpm at 4°C in a Beckman SW41 rotor. After fraction collection, cholesterol was measured enzymatically.

Enzyme Activity of Cholesterol 7 α -Hydroxylase and Sterol 27-Hydroxylase and Measurement of Liver Lipids

In livers from mice fed the high-cafestol or placebo diet for 3 weeks, enzyme activities of cholesterol 7 α -hydroxylase in microsomes and of sterol 27-hydroxylase in mitochondria were determined as described previously¹⁴ by measuring the mass conversion of cholesterol into 7 α - and 27-hydroxycholesterol. Liver lipids were measured as described.¹⁴

RNA Isolation, Blotting, and Hybridization Procedures

Isolation of total RNA and subsequent electrophoresis, Northern blotting, probe, and hybridization techniques were performed as described previously.^{5,15-17} The GAPDH mRNA or 18S rRNA was used as an internal standard to correct for differences in the amount of total RNA applied onto the gel. mRNA levels were quantified as described previously.¹⁴

Fecal Sterol Analysis

Feces were sampled for 3 days continuously in weeks 3 and 6 of the experimental period. Aliquots of lyophilized feces were used for determination of neutral and acidic sterol contents by gas-liquid chromatography procedures described previously.^{18,19}

In Vivo Hepatic VLDL Production in ApoE*3-Leiden Mice

Mice that were fed the high-cafestol or placebo diet for 3 weeks were fasted for 4 hours (from 8 AM to 12 noon) and then injected in the tail vein with 0.1 mL of PBS containing 100 μ Ci of Tran³⁵S label (ICN) to measure de novo apoB synthesis. After 30 minutes, Triton WR1339 (500 mg/kg body weight) was injected into the tail vein. Triton virtually completely inhibits serum VLDL clearance.²⁰ Serum triglycerides were determined before injection (t=0 minutes) and at different time points after injection. The hepatic VLDL triglyceride production rate was calculated from the slope of the curve. Serum collected 180 minutes after Triton injection was pooled, and VLDL was subsequently isolated by ultracentrifugation in triplicate. Triglycerides, total and free cholesterol, and phospholipids were measured enzymatically as described previously.¹² Cholesteryl esters were calculated as the difference between total and free cholesterol. ³⁵S-labeled apoB was precipitated by incubating 1.2 mL of the isolated VLDL fraction together with 0.1 mL of human LDL (100 μ g) as the carrier and 0.5 mL of isopropanol for 1 hour at room temperature and centrifuged for 10 minutes at 13 000 rpm in an Eppendorf centrifuge. The pellet was dissolved in 20% (wt/vol) SDS for 15 minutes at 60°C, and radioactivity was subsequently determined. To determine the apoB content of the VLDL particle, VLDL protein was subjected to 4% to 20% SDS-polyacrylamide gel electrophoresis, and the contribution of apoB to total protein was calculated.

Statistical Analyses

We calculated the change in serum lipids per mouse by subtracting values at the start of the experimental period from values obtained during the experimental period. After checking for normality, differences in changes between treatment groups and the control group were tested by using the 1-tailed, Student's unpaired *t* test. Other data were analyzed statistically by using a 2-tailed, Student's unpaired *t* test with the level of significance selected at $P<0.05$. Values are expressed as mean \pm SD.

Results

Food, Body Weight, and Alanine Aminotransferase

The initial body weight values (mean, 20.9 \pm 0.9 g) did not differ between treatment groups, whereas the final body weight values were significantly ($P<0.05$) lower in the high-cafestol treatment group compared with the placebo treatment group (21.0 \pm 0.2 versus 22.9 \pm 0.7 g). Average

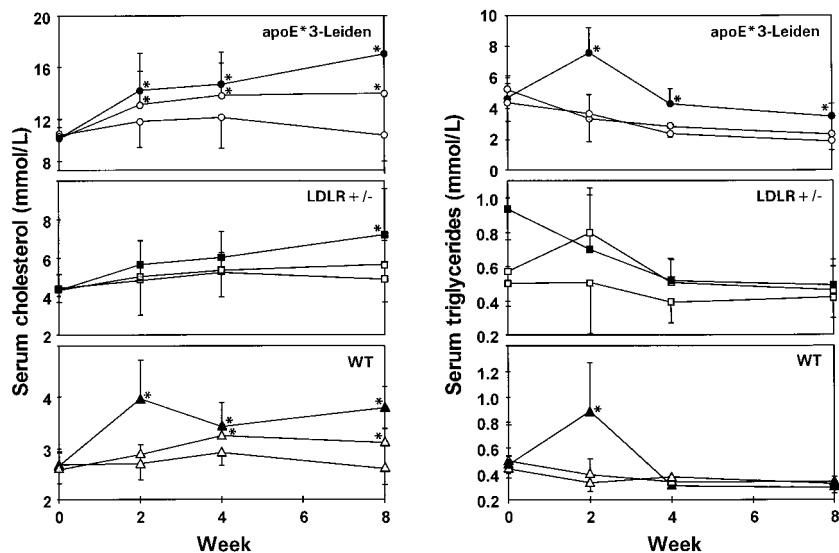


Figure 1. Effect of a high-cafestol (0.05% wt/wt) diet (black symbols), a low-cafestol (0.01% wt/wt) diet (gray symbols), and a placebo (white symbols) diet on serum cholesterol and triglycerides in apoE*3-Leiden (circles), LDLR +/- (squares), and WT (triangles) mice. Significant differences between the cafestol and placebo treatment are indicated by an asterisk.

daily food intakes were significantly ($P < 0.05$) lower (-7% and -15% , respectively) on the low-cafestol and high-cafestol diets compared with placebo treatment (mean, 2.7 ± 0.2 g/d). Concentrations of alanine aminotransferase did not significantly increase during consumption of cafestol in all mouse strains.

Cafestol Increases Serum Lipid and Lipoprotein Levels

Cafestol raised serum cholesterol in all 3 mouse strains after 8 weeks of dietary intervention (Figure 1). In apoE*3-Leiden mice, serum cholesterol was raised by 33% (3.46 mmol/L; 95% CI, 1.62 to 5.30) in the low-cafestol-diet group and by 61% (6.35 mmol/L; 95% CI, 4.47 to 8.22) in the high-cafestol-diet group. In LDLR +/- mice, serum cholesterol was raised by 20% (0.85 mmol/L; 95% CI, -0.25 to 1.94) in the low-cafestol-diet group and by 55% (2.37 mmol/L; 95% CI, 0.73 to 4.01) in the high-cafestol-diet group. In WT mice, serum cholesterol was raised by 24% (0.62 mmol/L; 95% CI, 0.34 to 0.90) in the low-cafestol-diet group and by 46% (1.21 mmol/L; 95% CI, 0.92 to 1.21) in the high-cafestol-diet group. The rise in serum cholesterol was predominantly due to a rise in VLDL and IDL cholesterol (Figure 2). Serum triglycerides were increased after 2 weeks in apoE*3-Leiden and WT mice and remained significantly higher in the apoE*3-Leiden mice during cafestol treatment compared with placebo treatment (Figure 1). Because the effects on serum cholesterol were most pronounced in the apoE*3-Leiden mice, we investigated the mechanism of the cholesterol-raising effect of cafestol in these mice.

Cafestol Decreases Hepatic Enzymes in Bile Acid Synthesis and Fecal Excretion of Bile Acids

To validate the effects of cafestol on bile acid synthesis obtained in cultured rat hepatocytes,⁵ we determined the effect of a high-cafestol diet on enzymes involved in bile acid synthesis and on fecal bile acid excretion in apoE*3-Leiden mice. Cafestol decreased cholesterol 7 α -hydroxylase activity and mRNA levels by 57% and 58%, respectively (Table 1). Cafestol also decreased sterol 27-hydroxylase mRNA levels by 32%, while the enzyme activity was paradoxically in-

creased by 40% (Table 1). The oxysterol formed by sterol 27-hydroxylase can be further converted by oxysterol 7 α -hydroxylase, an important enzyme in the alternate pathway of bile acid synthesis.²¹⁻²³ We measured mRNA levels of oxysterol 7 α -hydroxylase to investigate whether cafestol had other effects on the alternate pathway. The expression of this enzyme was decreased by 58% (Table 1).

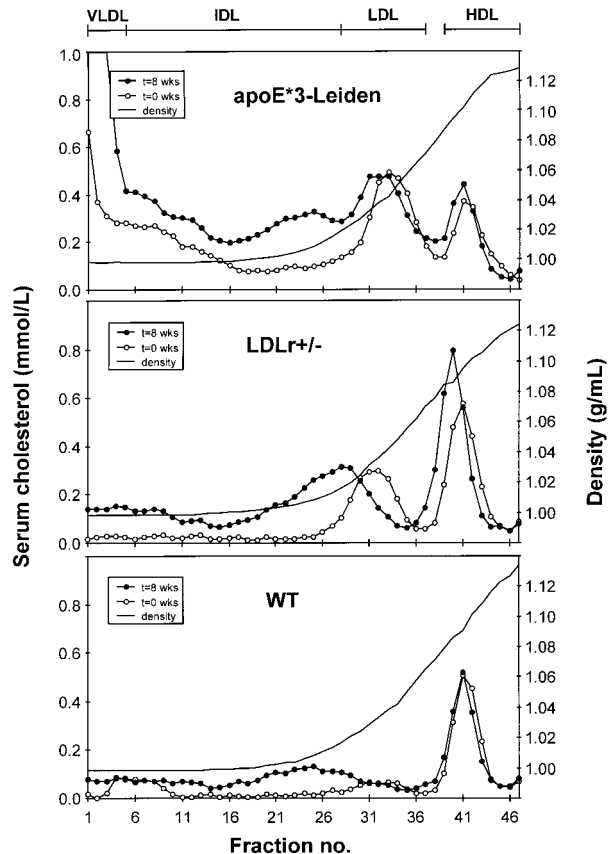


Figure 2. Effect of a high-cafestol (0.05% wt/wt) diet on cholesterol profiles in apoE*3-Leiden, LDLR +/-, and WT mice. Cholesterol profiles from pooled sera of 8 mice at the start of the experimental period (t=0, open symbols) and after 8 weeks (closed symbols) of treatment with the high-cafestol diet.

TABLE 1. Effect of Cafestol on Hepatic mRNA and Activity Levels in ApoE*3-Leiden Mice

	Placebo Diet, % of Placebo		High-Cafestol Diet, % of Placebo	
	Activity	mRNA	Activity	mRNA
Cholesterol 7 α -hydroxylase	100 \pm 5	100 \pm 41	43 \pm 1*	42 \pm 7*
Sterol 27-hydroxylase	100 \pm 10	100 \pm 12	140 \pm 5*	68 \pm 17*
Oxysterol 7 α -hydroxylase	ND	100 \pm 21	ND	41 \pm 16†
LDLR	ND	100 \pm 26	ND	63 \pm 14*

ND indicates not determined. Hepatic enzyme activities and mRNA levels of apoE*3-Leiden mice treated for 3 weeks with a high-cafestol (0.05% wt/wt) or placebo diet were determined after a 4-hour fasting period from 8 AM until 12 noon. Absolute activities of cholesterol 7 α -hydroxylase and sterol 27-hydroxylase from mice treated the placebo diet were 1.83 and 1.76 nmol \cdot h⁻¹ \cdot mg protein, respectively. Data shown are mean \pm SD (n=4 per group). A significant difference is indicated (* P <0.05, † P <0.005).

Because downregulation of enzymes involved in bile acid synthesis has consequences for the overall process of bile acid production, the amount of total and individual fecal bile acids was measured. Cafestol decreased the total amount of bile acids excreted in the feces by 41% (Table 2). Furthermore, the amount of fecal neutral sterols excreted after cafestol treatment appeared to be slightly lower compared with the placebo group (Table 2). These effects were similar after 3 and 6 weeks on the high-cafestol or placebo diet.

Effect of Cafestol on VLDL Production and Hepatic Lipid Metabolism

To investigate the effects of decreased bile acid synthesis on hepatic lipid metabolism, we determined the amount of hepatic lipids in apoE3*-Leiden mice treated with a high-cafestol or placebo diet for 3 weeks. Cafestol significantly (P <0.05) decreased the hepatic triglyceride content by 52% (86.3 \pm 10.6 versus 41.1 \pm 8.7 μ g/mg protein), but it did not alter the liver free and esterified cholesterol levels (data not shown). Although the hepatic content of free cholesterol in apoE*3-Leiden mice was apparently not affected by cafestol, the putative regulatory pool of cholesterol was increased, as indicated by a decreased expression of the LDLR (-37%; Table 1).

Because the excess hepatic cholesterol was not excreted into the bile, it might have been secreted into VLDL particles. Therefore, we measured nascent VLDL production in apoE*3-Leiden mice after 3 weeks on a high-cafestol or placebo diet. The VLDL apoB production was 2-fold de-

creased after cafestol treatment, indicative of a decline in secretion of VLDL particles (Figure 3A). However, the absolute number of cholesteryl esters in the VLDL particles during cafestol treatment was 4 times higher compared with placebo treatment (Table 3), indicative of the secretion of a β -VLDL-like particle, and resulting in a net 2-fold increased secretion of cholesteryl esters. The VLDL triglyceride production rate decreased to the same extent as the decline in apoB secretion (35.1 \pm 13.8 after cafestol treatment versus 63.1 \pm 17.5 μ mol \cdot h⁻¹ \cdot kg⁻¹ after placebo treatment; Figure 3B). Therefore, we did not observe a significant change in the absolute amount of triglycerides in nascent VLDL.

Discussion

In this study, cafestol increased serum cholesterol levels in apoE*3-Leiden, LDLR+/-, and WT mice, mainly in the VLDL and IDL fractions. In apoE*3-Leiden mice, cafestol decreased bile acid synthesis, as reflected by a reduction in the total amount of fecal bile acids, by downregulating the expression of enzymes involved in the neutral as well as in the alternate bile acid synthetic pathway. The decrease in bile acid synthesis resulted in a decline in LDLR mRNA levels and an increased secretion of VLDL cholesterol ester.

In contrast to other animal models,⁶⁻¹⁰ these mice are the first animals to show an increase in serum cholesterol due to cafestol that is similar to what has been observed in humans, making the mouse a good model to investigate the biochemical background of the cholesterol-raising effect of cafestol in humans. It should be noted that the increase in serum

TABLE 2. Effects of Cafestol on Fecal Bile Acid Excretion and Composition and on Excretion of Neutral Sterols From ApoE*3-Leiden Mice

Treatment	Bile Acid Excretion, μ mol \cdot d ⁻¹ \cdot 100 g ⁻¹ Body Weight									Neutral Sterol Excretion, μ mol \cdot d ⁻¹ \cdot 100 g ⁻¹ Body Weight
	DC	C	LC	CDC	HDC	UDC	α/β MC	ω MC		
Placebo diet	6.8 \pm 0.7 (100%)	21	15	4	1	2	3	28	26	34.3 \pm 1.3 (100%)
High-cafestol diet	4.0 \pm 0.1 (59%)	30	13	3	1	2	3	16	32	27.9 \pm 2.8 (81%)

DC indicates deoxycholate; C, cholate; LC, lithocholate; CDC, chenodeoxycholate; HDC, hyodeoxycholate; UDC, ursodeoxycholate; and MC, muricholate. Mice were treated with a high-cafestol diet or a placebo diet for 3 weeks. In feces, total bile acids, neutral sterols, and bile acid composition were determined as described in Methods. Data are mean \pm range of 2 individual feces samples of each group in percent. Values in parentheses represent percentages of the value obtained in animals treated with the placebo diet.

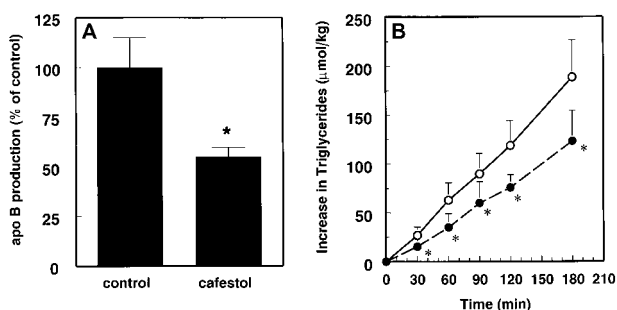


Figure 3. Effect of cafestol on VLDL production in apoE*3-Leiden mice. Tran³⁵S label (100 μ Ci) was injected into placebo- and cafestol- (0.05% wt/wt) treated apoE*3-Leiden mice ($n=8$ fasted mice per group). After 30 minutes Triton WR1339 (500 mg/kg body weight) was injected, and serum samples were taken at different time points. VLDL was isolated by ultracentrifugation from pooled sera collected 180 minutes after Triton injection. ³⁵S-labeled apoB was precipitated from the VLDL fraction, and radioactivity was subsequently measured (A). Serum triglyceride levels were determined at indicated time points and corrected for the triglyceride level at the time of injection. The values shown are mean \pm SD of 4 individual VLDL samples of each group (A) or mean \pm SD of serum samples of 8 mice (B). A significant difference between placebo- (open symbols) and cafestol- (closed symbols) treated mice is indicated by an asterisk ($P<0.05$).

cholesterol in humans is mainly present in LDL, whereas in mice, the rise was found predominantly in the VLDL-IDL range. Differences in the absorption and/or metabolism of coffee diterpenes, or in their effects on lipoprotein metabolism, may underlie the absence of a response in a range of other animal species.

Previously, we reported that cafestol suppressed bile acid synthesis by downregulation of cholesterol 7 α -hydroxylase and sterol 27-hydroxylase expression in rat hepatocytes.⁵ This concept is now confirmed in vivo in apoE*3-Leiden mice. Cafestol suppressed bile acid synthesis by downregulation of cholesterol 7 α -hydroxylase, sterol 27-hydroxylase, and oxysterol 7 α -hydroxylase expression, indicative of inhibition of both the acidic and the neutral pathways in bile acid synthesis. Whereas cafestol decreased mRNA levels of sterol 27-hydroxylase, its enzyme activity was paradoxically increased. An explanation for this apparent discrepancy would be that the enzyme oxysterol 7 α -hydroxylase is present in the mitochondrial fractions of these mice and converts 27-hydroxycholesterol into 7 α ,27-dihydroxycholesterol.^{21–23} Indeed, we found decreased expression of oxysterol 7 α -hydroxylase mRNA and that its expression is inhibited by cafestol. Thus, it is possible that the apparent increase in sterol 27-hydroxylase activity can be attributed to accumulation of its product, 27-hydroxycholesterol, caused by a

blockade in the subsequent metabolic conversion involving oxysterol 7 α -hydroxylase. Concomitant with the reduction in fecal bile acids, fecal excretion of neutral sterols tended to be lowered. However, because food intake in the cafestol-treated animals was slightly reduced, leading to a lesser intake of dietary cholesterol, the data on neutral sterol excretion may be an underestimate.

Theoretically, suppressed bile acid synthesis would increase the hepatic pool of free cholesterol. We did not find an effect on hepatic free cholesterol levels, but different metabolic pathways might have converted the free cholesterol into cholesteryl esters or removed it from the liver as such and/or via VLDL particles to maintain hepatic cholesterol homeostasis. Because we did not find a hepatic accumulation of cholesteryl esters, it appears plausible that the cholesterol that becomes available due to inhibition of bile acid synthesis is directly removed from the liver via VLDL particles (see below). In addition, high amounts of free cholesterol in cell membranes²⁴ may overshadow the subtle changes in free cholesterol caused by inhibition of bile acid synthesis. We found a substantial decrease in LDLR mRNA, which is a sensitive measure to detect changes in the putative regulatory pool of free cholesterol. Subtle increases in intracellular cholesterol prevent processing of the sterol regulatory element-binding protein, resulting in downregulation of LDLR gene transcription.²⁵ A similar decrease in LDLR mRNA levels has been shown in vitro in cultured rat hepatocytes⁵ and in HepG2 cells.²⁶ In contrast, divergent data were reported in other cell types,^{27,28} possibly because of different metabolic functions of these cells. Our results plead in favor of the hypothesis that the cholesterol-raising effect of cafestol can be explained by a reduced expression of the LDLR.

The rise in serum cholesterol during cafestol treatment may also be partly explained by an increased secretion of cholesteryl esters in VLDL. Despite the 2-fold reduction in VLDL particles, the 4-fold increase in the absolute amount of cholesteryl esters in VLDL particles, leading to a net 2-fold-enhanced secretion of cholesteryl esters during cafestol treatment, also contributed to the increase in plasma cholesterol levels after cafestol treatment. Concomitantly, the absolute amount of triglycerides in the particles did not change significantly. This was the result of a similar reduction in the VLDL triglyceride secretion rate and the secretion of VLDL particles. The decrease in VLDL triglyceride production rate and the reduced hepatic triglyceride content suggest impaired triglyceride synthesis. Whether this is due to a direct or indirect effect of cafestol on the activity or expression of the enzymes involved in triglyceride synthesis awaits further investigation.

TABLE 3. Effect of Cafestol on VLDL Composition in ApoE*3-Leiden Mice

Treatment	Triglycerides	Free Cholesterol	Cholesteryl Ester	Phospholipids
Placebo	26.5 \pm 6.0	8.7 \pm 0.9	11.8 \pm 3.8	12.4 \pm 2.2
Cafestol	18.8 \pm 3.0	9.9 \pm 1.2	48.1 \pm 23.7*	11.4 \pm 1.5

VLDL was isolated from the pooled sera of 8 fasted mice treated with a high-cafestol or a placebo diet. The serum was collected 180 minutes after Triton injection. Triglycerides, total cholesterol, free cholesterol, and phospholipids were measured enzymatically, and the amount of cholesteryl ester was calculated (see Methods). Apo B was also measured as indicated in Methods. Data are mean \pm SD of 3 individual VLDL samples from each group and are in μ mol/mg of apo B. A significant difference is indicated * $P<0.05$.

In conclusion, we found that cafestol inhibits bile acid synthesis by downregulation of both the neutral and the acidic pathway, leading to a decrease in expression of the LDLR and an elevated secretion of cholesteryl esters in VLDL. Suppression of bile acid synthesis may provide an explanation for the cholesterol-raising effects of unfiltered coffee in humans.

Acknowledgments

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