The coffee diterpene cafestol increases plasma triacylglycerol by increasing the production rate of large VLDL apolipoprotein B in healthy normolipidemic subjects[1–3]

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ABSTRACT

Background: Cafestol is a diterpene in unfiltered coffee that raises plasma triacylglycerol in humans.

Objective: We studied whether cafestol increases plasma triacylglycerol by increasing the production rate or by decreasing the fractional catabolic rate of $VLDL_1$ [Svedberg flotation unit (S_f) 60–400] apolipoprotein (apo) B. In addition, we studied the effect of cafestol on the composition of $VLDL_1$ and $VLDL_2$ (S_f 20–60).

Design: Eight healthy normolipidemic men were administered a daily dose of 75 mg cafestol for 2 wk. A bolus injection of 7 mg L-[5,5,5-$^2H_3$]leucine/kg body wt was given after a baseline period with no cafestol and again after treatment with cafestol. We derived kinetic constants to describe the metabolism of $VLDL_1$ apo B by using a multicompartmental model.

Results: Cafestol significantly increased plasma triacylglycerol by 31% or 0.32 mmol/L (95% CI: 0.03, 0.61); the increase was due mainly to a nonsignificant rise in $VLDL_1$ triacylglycerol of 57% or 0.23 mmol/L (95% CI: −0.02, 0.48). Cafestol significantly increased the mean rate of $VLDL_1$ apo B production by 80% or 755 mg/d (95% CI: 0.2, 5353), whereas it did not significantly change the mean fractional catabolic rate of $VLDL_1$ apo B (mean increase of 3 pools/d; 95% CI: −4, 10]). Cafestol did not change the composition of $VLDL_1$. A significant increase in the ratio of $VLDL_2$ cholesteryl ester to triacylglycerol indicates that $VLDL_2$ became enriched with cholesteryl esters at the cost of triacylglycerol.

Conclusion: Cafestol increases plasma triacylglycerol by increasing the production rate of $VLDL_1$ apo B, probably via increased assembly of $VLDL_1$ in the liver. *Am J Clin Nutr* 2001;73:45–52.

KEY WORDS Healthy men, cafestol, $VLDL_1$ apolipoprotein B, catabolism, VLDL composition, unfiltered coffee, cafetière coffee, French-press coffee, triacylglycerol, triglyceride

INTRODUCTION

Unfiltered coffee brews such as Scandinavian-type boiled coffee, cafetière (or French-press) coffee, and Turkish coffee raise plasma triacylglycerol and LDL-cholesterol concentrations in humans (1). Elevated concentrations of plasma triacylglycerol and LDL cholesterol are important risk factors for the development of cardiovascular diseases (2, 3). The responsible compound is cafestol, a lipid-soluble diterpene present in coffee beans (4). Unfiltered coffee brews contain 3–6 mg of this diterpene per cup (5). Filtered coffee does not contain cafestol because the diterpene is retained by a paper filter (6). The results of experimental and epidemiologic studies suggest that a high intake of unfiltered coffee is associated with an increased incidence of cardiovascular disease (7–9).

In human subjects, the effects of cafestol on plasma triacylglycerol and on LDL cholesterol differ in their kinetics. When 22 volunteers consumed 0.9 L cafetière coffee per day, plasma triacylglycerol increased by a maximum of ≈30% after 2–4 wk. The effect on triacylglycerol was transient because concentrations were back at baseline after 24 wk (10). The onset of the rise in LDL cholesterol was much slower: a maximum increase of ≈12% was reached only after 8–12 wk of cafetière coffee consumption. The rise in LDL cholesterol is not transient; epidemiologic studies showed that chronic consumers of boiled coffee have permanently elevated plasma cholesterol concentrations (11–13). Because the rise in triacylglycerol is the first step in a cascade of changes in lipoprotein metabolism with consumption of cafestol (1, 10), this rise might play a role in the subsequent increase in LDL-cholesterol concentrations.

In this study, we examined the mechanism by which cafestol increases plasma triacylglycerol in normolipidemic men. In humans, most triacylglycerol is transported in VLDL particles. However, VLDL can be fractionated into 2 metabolically discrete components: $VLDL_1$ (S_f 60–400) and $VLDL_2$ (S_f 20–60). Compared with $VLDL_1$, $VLDL_2$ particles are smaller,

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enriched in cholesteryl ester, depleted in triacylglycerol, and have a lower ratio of apolipopotein (apo) E and apo C to apo B (14). There is evidence of independent regulation of the secretion of $VLDL_1$ and $VLDL_2$ by the liver (15–18). High concentrations of triacylglycerol-rich $VLDL_1$ have been suggested to give rise to a preponderance of small, dense LDL. Small LDL particles appear to be a higher risk factor for the development of cardiovascular diseases than are larger, more buoyant LDL particles (14). Because $VLDL_1$ carries almost one-half of the total amount of triacylglycerol in the circulation, whereas $VLDL_2$ carries only about one-fifth, we used a modeling approach to quantify the kinetic properties of $VLDL_1$ apo B metabolism (19). Our hypothesis was that either increased secretion of $VLDL_1$ into the circulation—ie, an increased production rate—or a decreased clearance of $VLDL_1$ particles—ie, a decreased fractional catabolic rate—might cause a rise in $VLDL_1$ and thus in plasma triacylglycerol. Alternatively, an enrichment of $VLDL_1$ or $VLDL_2$ particles with triacylglycerol might also contribute to the triacylglycerol-raising effects of cafestol. Therefore, we determined the effects of cafestol on $VLDL_1$ and $VLDL_2$ composition.

SUBJECTS AND METHODS

Subjects

Fifty-two men entered a medical screening. All had normal hepatic and renal function, and none had hematologic abnormalities. No subject was taking any medication known to affect lipid metabolism. Eight healthy normolipidemic men with plasma triacylglycerol concentrations between 0.9 and 2.0 mmol/L were selected to participate in the study. They had a mean (±SEM) age of 21 ± 1 y and a body mass index (in kg/m^2) of 22.30 ± 0.65. Three volunteers had the apo E4,E3 phenotype, 3 the apo E3,E3 phenotype, and two the apo E3,E2 phenotype. The first 2 subjects were studied at the Department of Pathological Biochemistry, Glasgow Royal Infirmary, Glasgow, Scotland. The remaining 6 subjects were studied at the Department of Internal Medicine, Nijmegen Academic Hospital, Nijmegen, Netherlands. The subjects were well informed about the purpose and protocol of the experiment. They gave their written, informed consent before the start of the study.

Preparation of the cafestol supplements and the tracer

Four kilograms of ground coffee beans was solubilized in 4 L diisopropylether. Five hundred grams of the extracted coffee oil was saponified under nitrogen gas for 2 h at room temperature with 300 mL methanolic KOH (100:5, vol:wt). After the addition of 750 mL heptane, the cafestol-kahweol mixture was extracted once with 500 mL methanol:water (112.5:10, by vol) and twice with 500 mL methanol:water (118.8:5, by vol). The 3 extracts were combined, dried to 300 g under vacuum, and saponified for 30 min at 40°C with 25 g KOH dissolved in 16 mL H_2O and 48 mL methanol. Five hundred milliliters water was added and the cafestol-kahweol alcohols were extracted once with 900 mL and 4 times with 500 mL dichloromethane:methanol (87.3:12.7, by vol). The combined extracts were evaporated to dryness under vacuum, dissolved in 125 mL ethylacetate, and shaken with 7.5 g active carbon for 1 h at room temperature. The mixture was filtered and the filtrate evaporated to dryness under vacuum. To purify the cafestol-kahweol alcohol mixture, the dried residue was dissolved in 200 mL hot methanol and cooled to 4°C to crystallize the cafestol-kahweol alcohols. The supernate was evaporated to dryness under vacuum, dissolved in a smaller volume of hot methanol, and again cooled to 4°C.

Pure cafestol was obtained by hydrogenation of the kahweol in the diterpene mixture. Twenty grams of the cafestol-kahweol alcohol mixture was dissolved in 0.5 L 96% ethanol by using 4.2 g $PdPbCaCO_3$ as a catalyst. A total of 600 mL H_2 was stirred vigorously through the cafestol-kahweol alcohol mixture for 2.25 h at room temperature. The solution was filtered to remove the catalyst. The filtrate was dissolved in 100 mL water and allowed to stand overnight at 4°C. The precipitate was dried under vacuum, dissolved in 200 mL hot methanol, and cooled to 4°C to crystallize the cafestol alcohol.

After being dried under vacuum, the cafestol alcohol was reesterified to palmitate by the following procedure. At 40–50°C and under nitrogen gas, 20 g of the cafestol alcohol mixture was acetylated by adding 400 mL diisopropylether and 40 g pyridine. When cool, 36 mg palmitylchloride was added and the mixture was extracted with 280 mL of 2 mol HCl/L. The organic phase was extracted twice with 280 mL H_2O. The extracts were cooled to 4°C and filtered. The filtrate was evaporated to dryness under vacuum. After solubilization in 200 mL hexane, the cafestol palmitate was purified by elution from a chromatographic column containing 250 g aluminum oxide 90 (catalog no. 1.01077.1000; Merck, Amsterdam) in hexane with 1000 mL hexane. The resulting 32 g cafestol palmitate mixture had a purity of 85%, as determined by gas chromatography–mass spectrometry (GC-MS) (5). The impurities consisted of free cafestol, cafestol dipalmitate, and palmitic acid.

Cafestol palmitate was dissolved in a mixture of sunflower oil and palm oil (3:2, by mass). Earlier studies showed that this mixture of sunflower oil and palm oil has no effect on plasma cholesterol concentrations. The daily amount of cafestol provided in this study, ie, 75 mg cafestol or 131.5 mg cafestol palmitate (which is equivalent to the consumption of ≈15 cups of unfiltered coffee per day), was divided between 2 capsules to spread the cafestol intake over the day. This amount of cafestol was chosen to induce a significant increase in plasma triacylglycerol in 8 healthy subjects on the basis of data from previous intervention studies (1).

L-[5,5,5-$^2H_3$]Leucine (d_3-leucine) with a purity >99% was purchased from Cambridge Isotope Laboratories (Woburn, MA). It was dissolved in 0.9% saline solution to a concentration of 10 g/L, sterilized, and checked for pyrogens.

Study design

Plasma lipids, plasma $VLDL_1$ apo B, $VLDL_2$ apo B, the production and fractional catabolic rates of $VLDL_1$ apo B, and the composition of $VLDL_1$ and $VLDL_2$ were determined after a cafestol-free baseline period and directly after 2 wk of treatment with cafestol. We first performed a VLDL turnover study to obtain baseline values. For this, subjects fasted for 12 h overnight. At ≈0800, the tracer d_3-leucine was administered as an intravenous bolus injection (7.0 mg/kg body wt). We took plasma samples immediately before tracer injection and at multiple time points throughout the following 48 h: at 2, 5, 10, 15, 20, 30, 40, 60, 80, and 120 min, and at 3, 4, 6, 8, 10, 11, 14, 24, 36, and 48 h. A total of 240 mL blood was sampled in each VLDL turnover study. To keep the chylomicron production minimal during the initial 12-h phase of the turnover, participants fasted until 1800 and were then permitted a light, fat-free meal. Blood samples taken after 24 and 48 h were also taken in the

fasting state. At least 2 wk after the first turnover experiment, subjects swallowed 2 capsules with 37.5 mg cafestol each per day for a period of 2 wk. On the last 2 d of this 2-wk treatment period, a second VLDL turnover study was performed as described above. The 16 VLDL turnover studies were done over a period of 9 mo. We always performed 2 turnover studies at the same time; a VLDL turnover study after a cafestol-free baseline period in one subject was combined with a VLDL turnover study after treatment with cafestol in a second subject. This reduced the effect of drifts in the variables of interest with time.

The subjects did not drink any coffee other than filtered coffee for ≥2 mo before the start of the experiment. In addition, subjects were not allowed to drink any coffee brews other than drip filter coffee, percolated coffee, or instant coffee throughout the study. Subjects were asked to maintain their usual pattern of diet and physical activity during the entire experimental period. Alcohol consumption was restricted to a maximum of 2 units (20 mL) per day. The subjects were asked to record any deviation from their usual diet and physical activity in a special diary. In addition, subjects were asked to record the time they consumed the cafestol capsules, the daily alcohol intake, signs of illness, and medications used.

Plasma lipids, alanine aminotransferase, and apo E phenotype

Plasma concentrations of cholesterol, triacylglycerol, and alanine aminotransferase were determined in fasting subjects. We measured plasma cholesterol and triacylglycerol by enzymatic methods (CHOD-PAP and GPO-PAP, catalog nos. 237574 and 701912, respectively; Boehringer Mannheim, Mannheim, Germany). HDL cholesterol was determined by a precipitation method using phosphotungstic-Mg^{2+} (20). LDL cholesterol was calculated as described by Friedewald et al (21). We isolated total VLDL by ultracentrifugation of plasma for 16 h at 168 000 × *g* (36 000 rpm) and 4 °C using the TFT 45.6 fixed angle rotor (Kontron, Zürich, Switzerland) in a Beckman L7-55 ultracentrifuge (Beckman, Palo Alto, CA) (22). In this VLDL fraction, we analyzed total cholesterol and triacylglycerol as described above. To assess the effect of cafestol on hepatic functioning, plasma activity of alanine aminotransferase was determined with a Hitachi 747 analyzer (Roche, Almere, Netherlands). Apo E phenotypes were determined after isoelectric focusing of VLDL apos, as described previously (23). Results were periodically checked with apo E genotyping according to Hixson and Vernier (24).

Lipoprotein composition and apo B pool size

We isolated VLDL$_1$ and VLDL$_2$ from 2 mL plasma by cumulative flotation gradient ultracentrifugation using a 6-step discontinuous salt gradient (25, 26). The lipid composition of the 2 VLDL subfractions was determined by using the methods mentioned above for total cholesterol and triacylglycerol determination. We used enzymatic reagents for the determination of free cholesterol and phospholipids (catalog nos. 310328 and 691844, respectively; Boehringer Mannheim). The amount of cholesteryl esters was subsequently calculated as the difference between total and free cholesterol. To correct for losses of lipids and protein during ultracentrifugation, we multiplied the measured content of lipids and protein in both VLDL subfractions with the ratio of VLDL triacylglycerol to (VLDL$_1$+VLDL$_2$) triacylglycerol. The mean (±SEM) recovery of VLDL$_1$+VLDL$_2$ triacylglycerol compared with VLDL triacylglycerol was 70 ± 6% after the cafestol-free baseline period and 64 ± 9% after treatment with cafestol. We assumed that the percentage loss of VLDL triacylglycerol was approximately equal for both VLDL subfractions because they were isolated in a similar manner.

We precipitated apo B from both VLDL subfractions by adding an equal volume of isopropanol (27). We determined the apo B content of VLDL$_1$ and VLDL$_2$ as the difference between total and isopropanol-soluble protein (25, 27, 28). To correct for losses of apo B during ultracentrifugation, we multiplied the measured apo B content by the ratio of VLDL triacylglycerol to (VLDL$_1$+VLDL$_2$) triacylglycerol as described above. VLDL$_1$ and VLDL$_2$ apo B pool sizes were calculated as the product of plasma volume (assumed to be 4% of body weight) and the plasma concentration of apo B in VLDL$_1$ and VLDL$_2$. The leucine content of the apo B pools was assumed to be 12.12% of apo B (29).

d_3-Leucine enrichment in apo B

The precipitated apo B from the VLDL$_1$ fraction was delipidated with ethanol:ether (3:1) and dried with ether. We subsequently hydrolyzed apo B with 1.0 mL of 6 mol HCl/L at 110 °C for 24 h (25).

From plasma, we precipitated proteins with trichloroacetic acid as described previously (19). We determined the enrichment of leucine with d_3-leucine (ie, the tracer-tracee ratio) in apo B hydrolysates and in plasma free amino acids by GC-MS (MD800; Finnigan, Manchester, United Kingdom) (19). The maximal level of enrichment of leucine with d_3-leucine was approximately similar in all turnovers. The mean tracer-tracee curves are shown in **Figure 1** (upper panel). Tracer mass was calculated by multiplying the tracer-tracee ratios by the tracee mass (Figure 1, lower panel).

Kinetic analysis and multicompartmental modeling

We adapted a model (**Figure 2**) that was developed and described previously (19). The first 4 compartments explain the kinetics of plasma leucine. d_3-Leucine is injected into the plasma compartment (compartment 1). An intracellular pool in the liver (compartment 2) is the immediate precursor of the d_3-leucine. Compartments 3 and 4 represent body protein with a slow turnover. Input of leucine into VLDL$_1$ apo B occurs from compartment 2 via a delay compartment (compartment 5). The delay indicates the time between the injection of d_3-leucine in the plasma compartment and its appearance in VLDL$_1$ apo B. The delay was set at 0.5 h initially but was adjusted between 0.4 and 0.6 h when required to obtain a better fit of the curves. Input of apo B into VLDL$_1$ occurred in compartment 6. Compartments 6 and 7 represent a delipidation chain. Compartment 8 represents a remnant particle that does not undergo further delipidation but is eventually eliminated from the plasma. In our patients with low plasma triacylglycerol and a high turnover rate of VLDL$_1$ particles, we could not obtain a satisfactory fit for the VLDL$_2$ apo B data for 2 reasons. First, the experimental data for VLDL$_2$ apo B were imprecise, and second, our model could not clearly distinguish which part of the VLDL$_2$ apo B was obtained via delipidation from VLDL$_1$ and which part was directly synthesized by the liver. Therefore, we restricted the model to describe the kinetics of VLDL$_1$ apo B only.

Before fitting our model to the data, we introduced parameter dependencies to prevent the number of unknown parameters (transfer rate constants) from exceeding the number of equations. For the plasma kinetics, the transfer rate constants $k_{1,2}$ and $k_{2,1}$ were both fixed at 2.5, and the value for $k_{3,4}$ was set at

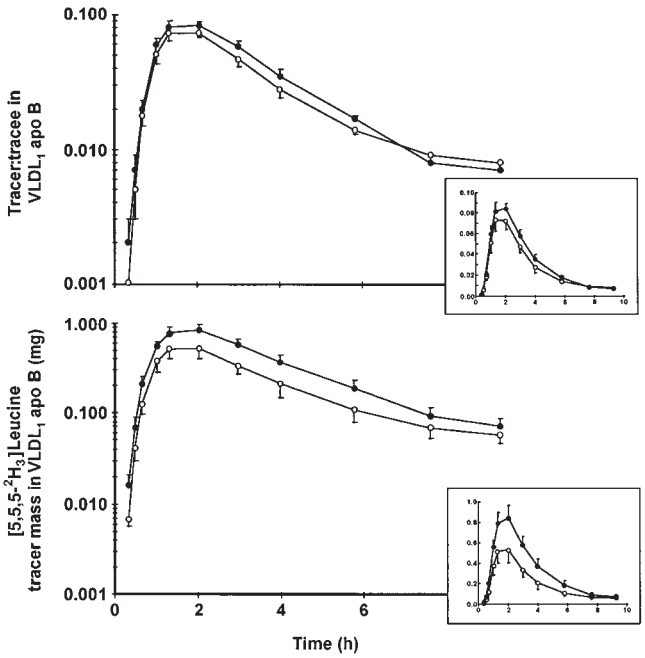

FIGURE 1. Mean (±SEM) ratio of tracer to tracee in $VLDL_1$ apolipoprotein (apo) B and incorporation of [5,5,5-$^2H_3$]leucine mass into $VLDL_1$ apo B after a baseline period with no cafestol treatment (○) or after a 2-wk treatment period with cafestol (●) in 8 normolipidemic men. [5,5,5-$^2H_3$]Leucine was given as a bolus infusion at time zero. Tracer mass was calculated by multiplying the tracer-tracee ratio (as determined by gas chromatography–mass spectrometry) by the tracee mass. In the inserts, the tracer-tracee ratio and tracer mass are depicted on a linear scale.

$0.1 \times k_{4,3}$. These values were determined in previous studies on long-term data in a large group of subjects (19). We assumed that the delipidation rate from compartments in $VLDL_1$ was similar. Therefore, the delipidation rate constants $k_{7,6}$ and $k_{0,7}$ were made equal. These parameter dependencies were applied in all 16 turnover studies.

The measured data, ie, the tracer-tracee ratios and the $VLDL_1$ apo B leucine masses, were analyzed with the SAAMII program (SAAM Institute, University of Washington, Seattle). This program derives the value of the kinetic rate constants that produce the best fit between the calculated and the measured tracer-tracee ratios. Initially, the tracer-tracee ratios were assigned an SD that was related to the precision of their measurement by GC-MS. Previous studies showed that GC-MS analysis of the tracer-tracee values produced a CV (or relative SD) of ≈1% (30). Thus, we assumed that the SD at the peak value of the tracer-tracee curve, which in our study was typically between 0.05 and 0.1, equalized 1% of that peak height. SAAMII was subsequently used to calculate a weight for each tracer-tracee ratio, which is the reciprocal of the square of the SD assigned to these data. During the fitting process, peak values of tracer-tracee ratios were assigned a lower SD, thus more weight, when this would lead to a better fit of the model to the data. The weights were adjusted until there was no further improvement in the sum of squares. Eventually, we applied a mean SD of $\approx 3 \times 10^{-5}$ to the peak ratios of $VLDL_1$ apo B leucine, which represented a CV of ≈0.1%. The derived kinetic constants and the transport rates were considered acceptable when the calculated curves fitted the observed data without systematic error and when the calculated masses for $VLDL_1$ apo B were within 10% of the measured apo B pool sizes.

The $VLDL_1$ apo B production rate (in mg $VLDL_1$ apo B/d) was calculated from the output from compartment 2 (ie, $k_{5,2} \times$ the mass of leucine in compartment 2). The $VLDL_1$ apo B fractional catabolic rate (in pools/d) was calculated by dividing the sum of outputs from compartments 7 and 8 (ie, $k_{0,7} \times$ the mass of leucine in compartment 7 and $k_{0,8} \times$ the mass of leucine in compartment 8; both expressed as mg leucine/d) by the $VLDL_1$ apo B mass (ie, the combined masses of leucine in compartments 6, 7, and 8).

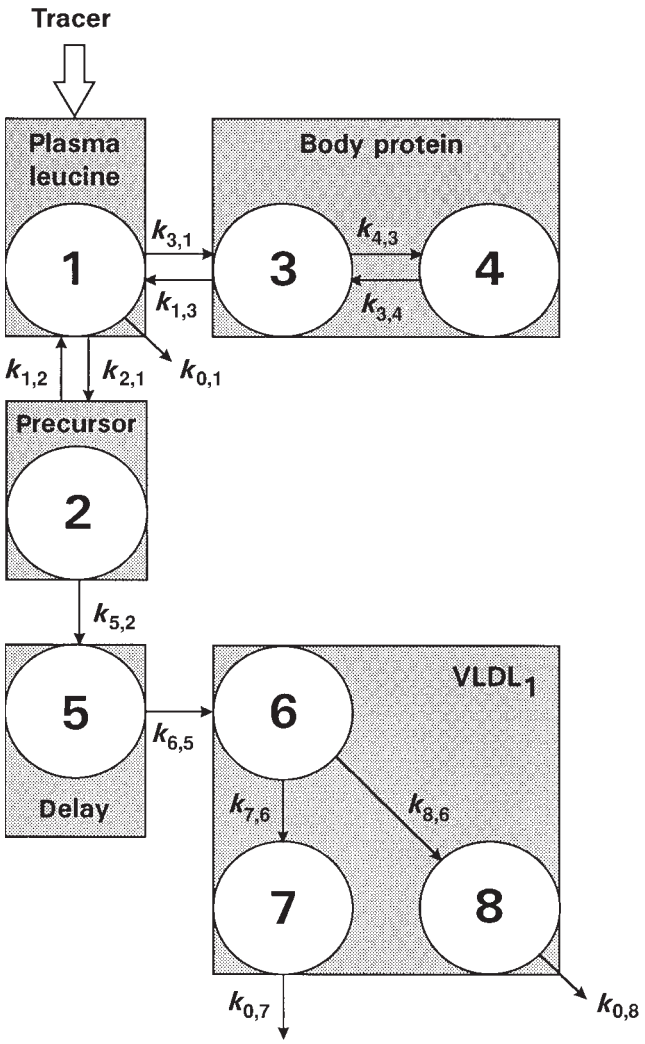

FIGURE 2. Multicompartmental model of apolipoprotein (apo) B metabolism. Bolus infusion data were analyzed with the same model in all 8 subjects. Plasma leucine (compartment 1) receives the [5,5,5-$^2H_3$]leucine tracer and distributes it to the body protein pools (compartment 3 and 4) and to the intracellular precursor pool for apo B synthesis (compartment 2). After the delay (compartment 5), the tracer appears in $VLDL_1$ via compartment 6, and then throughout delipidation in compartment 7, or throughout remnant formation in compartment 8. Parameter dependencies were $k_{2,1} = k_{1,2} = 2.5$; $k_{3,4} = 0.1 \times k_{4,3}$; $k_{7,6} = k_{0,7}$.

TABLE 1
Plasma lipids before and after a daily dose of 75 mg cafestol for 2 wk in healthy normolipidemic men

Plasma lipids	Baseline	Cafestol treatment	Change (95% CI)
		mmol/L	
Triacylglycerols	1.05 ± 0.08[1]	1.37 ± 0.16[2]	0.32 (0.03, 0.61)
VLDL triacylglycerols	0.61 ± 0.09	0.86 ± 0.18	0.25 (−0.15, 0.65)
VLDL$_1$ triacylglycerols	0.40 ± 0.05	0.63 ± 0.11	0.23 (−0.02, 0.48)
VLDL$_2$ triacylglycerols	0.21 ± 0.02	0.24 ± 0.03	0.02 (−0.04, 0.08)
Total cholesterol	4.08 ± 0.29	4.23 ± 0.22	0.15 (−0.06, 0.36)
VLDL cholesterol	0.40 ± 0.07	0.50 ± 0.08	0.10 (−0.07, 0.27)
LDL cholesterol	2.53 ± 0.30	2.62 ± 0.23	0.09 (−0.16, 0.34)
HDL cholesterol	1.16 ± 0.05	1.14 ± 0.05	−0.02 (−0.11, 0.07)

[1] $\bar{x}$ ± SEM; n = 8.
[2] Significantly different from baseline, $P < 0.05$ (Student's t test).

Statistical analysis

All data are expressed as means ± SEMs. We tested whether cafestol increased the VLDL$_1$ apo B production rate or decreased the VLDL$_1$ apo B fractional catabolic rate with a paired, two-tailed Student's t test. In addition, we tested whether cafestol affected the ratio of cholesteryl ester to triacylglycerol of VLDL$_1$ and VLDL$_2$ with a paired two-tailed Student's t test. The variables VLDL$_1$ apo B mass and VLDL$_1$ apo B production had a skewed distribution and were therefore logarithmically transformed before statistical comparison. Statistical analysis was performed with use of MINITAB (version 13; Minitab, Inc, Coventry, United Kingdom).

RESULTS

Plasma triacylglycerol, composition of VLDL$_1$ and VLDL$_2$, and plasma alanine aminotransferase

Cafestol treatment for 2 wk significantly increased average total triacylglycerol by 31% (**Table 1**). VLDL$_1$ triacylglycerol increased nonsignificantly by 57%, whereas VLDL$_2$ triacylglycerol increased nonsignificantly by only 11%. About 72% of the rise in plasma triacylglycerol was due to a rise in VLDL$_1$ triacylglycerol, and an additional 7% could be explained by a rise in VLDL$_2$ triacylglycerol.

Cafestol nonsignificantly increased the VLDL$_1$ apo B pool size by 59% (**Figure 3**). This increase was of the same magnitude as the increase in VLDL$_1$ triacylglycerol, suggesting that the composition of VLDL$_1$ was not altered (**Table 2**). Indeed, the VLDL$_1$ ratio of cholesteryl ester to triacylglycerol—an index of particle composition—was not significantly different after cafestol treatment (mean increase of 0.02; 95% CI: −0.09, 0.05).

Cafestol nonsignificantly increased the VLDL$_2$ apo B pool size by 31% or 32 mg (95% CI: −27, 90). This increase was larger than the increase in VLDL$_2$ triacylglycerol, suggesting that VLDL$_2$ was depleted in triacylglycerol. Indeed, the percentage cholesteryl ester in VLDL$_2$ increased (Table 2), which was reflected by a significantly higher VLDL$_2$ ratio of cholesteryl esters to triacylglycerol (mean increase of 0.12; 95% CI: 0.07,

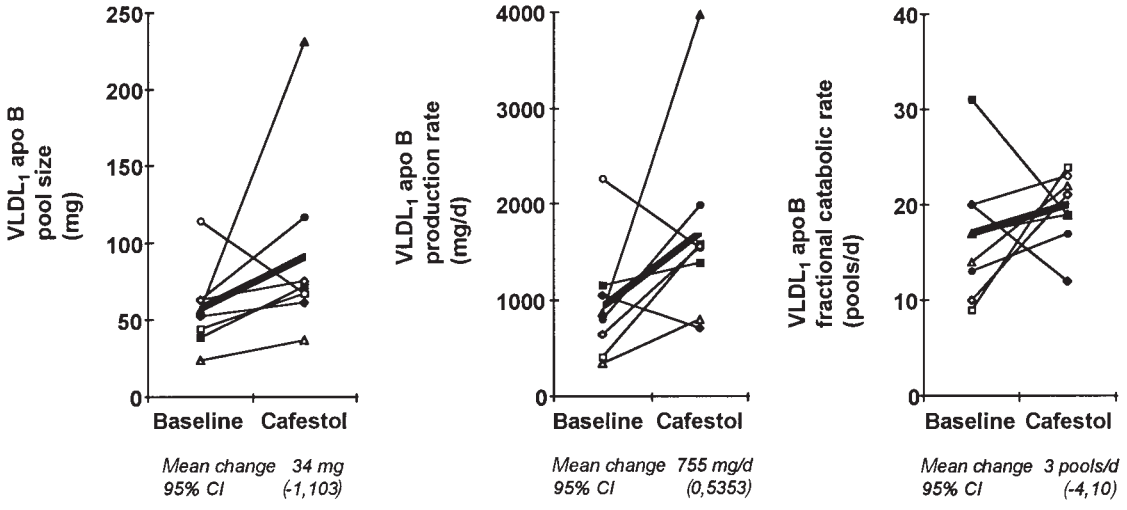

FIGURE 3. Individual changes in the pool size, the production rate, and the fractional catabolic rate of VLDL$_1$ apolipoprotein (apo) B after cafestol treatment for 2 wk. The heavy line indicates the mean change. The mean changes in VLDL$_1$ apo B pool size, production rate, and fractional catabolic rate after the results of one hyperresponding subject (▲) were rejected were 14 mg (95% CI: −0.72, 71.42), 418 mg/d (95% CI: −0.1, 3915), and 3 pools/d (95% CI: −5, 11), respectively.

TABLE 2
Effect of a daily dose of 75 mg cafestol for 2 wk on the composition of $VLDL_1$ and $VLDL_2$ in healthy normolipidemic men[1]

	Baseline		Cafestol treatment		Change	
	Concentration	Mass percent	Concentration	Mass percent	Concentration	Mass relative to apo B
	mg/L plasma	*% by mass*	*mg/L plasma*	*% by mass*	*mg/L plasma*	*mg/mg apo B*
$VLDL_1$						
Triacylglycerols	3.58 ± 1.29[2]	67	5.63 ± 2.85	66	2.05 ± 0.11	−0.12 ± 0.18
Free cholesterol	0.24 ± 0.21	5	0.42 ± 0.20	5	0.18 ± 0.09	0.05 ± 0.05
Cholesteryl esters	0.41 ± 0.30	8	0.64 ± 0.42	8	0.24 ± 0.15	−0.07 ± 0.08
Phospholipids	0.62 ± 0.35	12	1.02 ± 0.44	12	0.40 ± 0.16	0.02 ± 0.05
Protein	0.50 ± 0.22	9	0.81 ± 0.45	10	0.32 ± 0.19	—
$VLDL_2$						
Triacylglycerols	1.90 ± 0.61	47	2.10 ± 0.73	42	0.20 ± 0.29	−0.11 ± 0.06
Free cholesterol	0.29 ± 0.13	7	0.39 ± 0.16	8	0.09 ± 0.05	0.00 ± 0.01
Cholesteryl esters	0.62 ± 0.41	15	0.95 ± 0.51	19	0.33 ± 0.11	0.03 ± 0.02
Phospholipids	0.70 ± 0.22	17	0.94 ± 0.46	19	0.25 ± 0.14	−0.00 ± 0.03
Protein	0.54 ± 0.25	13	0.66 ± 0.24	13	0.12 ± 0.13	—

[1] Apo, apolipoprotein. No significant differences were observed before or after treatment with cafestol.
[2] $\bar{x}$ ± SEM; n = 8.

0.16). Cafestol nonsignificantly increased mean concentrations of plasma alanine aminotransferase from 20 to 33 U/L.

Production and fractional catabolic rate of $VLDL_1$ apo B

The tracer mass curves showed that cafestol treatment induced more tracer mass in $VLDL_1$ apo B because of a more rapid appearance (ie, a higher production rate), whereas this treatment induced only a minor increase in the clearance of the tracer from the circulation (Figure 1, lower panel). This suggested that cafestol increased the amount of $VLDL_1$ apo B mass, and thus the amount of $VLDL_1$ particles, mainly because of an increased production rate of $VLDL_1$ apo B.

Multicompartmental modeling of the tracer-tracee data showed that cafestol treatment elevated $VLDL_1$ apo B (Figure 1, lower panel) by significantly increasing the mean production rate of $VLDL_1$ apo B by 80% (Figure 3). Most subjects showed an increase in the $VLDL_1$ apo B production rate with consumption of cafestol. Cafestol nonsignificantly increased the mean fractional catabolic rate of $VLDL_1$ apo B by 17% (Figure 3). One subject appeared to be a hyperresponder to cafestol. However, we could find no reason based on his starting lipid values, his apo E3,E2 phenotype, or his baseline $VLDL_1$ kinetic parameters to consider him different from the other subjects. Data analysis was performed with and without the results of this subject (Figure 3); rejection of the results of this subject resulted in a nonsignificant increase in the mean $VLDL_1$ apo B production rate of 44% and in a nonsignificant increase in the mean $VLDL_1$ apo B fractional catabolic rate of 18%.

DISCUSSION

The results of this study indicate that in healthy normolipidemic subjects, a daily dose of cafestol equivalent to 15 cups of unfiltered coffee increased plasma triacylglycerol mainly by increasing the production rate of $VLDL_1$ apo B. This resulted in an increased amount of $VLDL_1$ particles in the circulation. Cafestol did not change the composition of $VLDL_1$. $VLDL_2$ became enriched with cholesteryl esters at the cost of triacylglycerol.

The secretion of apo B–containing lipoproteins appears to be regulated primarily at a posttranslational level because messenger RNA (mRNA) concentrations of apo B did not change in many situations in which the secretion of apo B from cultured liver cells was altered over a wide range. The amount of VLDL apo B reaching the circulation is therefore largely determined by the proportion that escapes degradation after the synthesis of the protein (31). Substances like oleate or insulin, which are known to affect the secretion of VLDL apo B without altering its concentrations of mRNA (32–34), might do this by increasing or decreasing the $VLDL_1$ assembly rate in the liver, which in turn affects the rate at which $VLDL_1$ apo B is secreted into the circulation. Therefore, cafestol might increase the rate at which $VLDL_1$ apo B is secreted into the circulation by increasing the assembly of apo B with lipids inside liver cells.

The assembly of apo B–containing lipoproteins by the liver is suggested to be a 2-step process. To initiate lipoprotein assembly, the N-terminal portion of apo B is thought to be translocated into the lumen of the rough endoplasmic reticulum, where it receives a small amount of lipid (whether this is cholesteryl ester or triacylglycerol is still a matter of debate) via the action of microsomal triacylglycerol transfer protein (MTP) (35, 36). If this addition of lipid does not occur, or if MTP is absent (37), apo B may be degraded. Thus, MTP appears to control the number of apo B lipoprotein particles secreted by the liver and thereby the apparent production rate of $VLDL_1$ apo B. Therefore, up-regulation of MTP activity is one possible explanation for increased secretion of $VLDL_1$ apo B with consumption of cafestol.

In the second step of VLDL assembly, the primordial lipoprotein coalesces with a large apo B–free droplet of triacylglycerol at the junction of the rough and smooth endoplasmic reticulum to form large triacylglycerol-rich VLDL (35, 36, 38). This second step depends on the availability of hepatic triacylglycerol (39). An alternative explanation for the action of cafestol is therefore that it might increase the availability of triacylglycerol in the liver by increasing the synthesis of triacylglycerol or by decreasing the rate of β-oxidation of fatty acids. The rate of β-oxidation of fatty acids is modulated by peroxisome proliferator activated receptor α activity, which, however, is not affected by cafestol (40). Therefore, the 2 most likely mechanisms for an effect of cafestol on $VLDL_1$ apo B production are that cafestol increases MTP action or that it increases the synthesis

of triacylglycerol in the liver. Both might lead to apo B being used for the production of $VLDL_1$ instead of being broken down.

Cafestol did not change the composition of $VLDL_1$, but $VLDL_2$ became enriched with cholesteryl esters at the cost of triacylglycerol. This was reflected in a significant increase in the $VLDL_2$ ratio of cholesteryl ester to triacylglycerol. Recently, a change in VLDL composition with cafestol treatment was also found in apo E*3-Leiden transgenic mice (41). VLDL particles became enriched with cholesteryl esters, suggestive of the secretion of a β-VLDL–like particle. In this transgenic mice species, cafestol significantly suppressed bile acid synthesis (41). We hypothesized that the cholesterol that becomes available in the liver because of inhibition of bile acid synthesis might be directly removed via secretion into VLDL particles. Availability of intrahepatic cholesterol might therefore be an important regulator of the secretion of $VLDL_2$ apo B.

In conclusion, our data indicate that cafestol raises plasma triacylglycerol predominantly through an increased production rate of $VLDL_1$ apo B after 2 wk of consumption. This results in a higher number of $VLDL_1$ particles in the circulation. It appears unlikely that this higher number of $VLDL_1$ particles is mainly responsible for the subsequent rise in LDL-cholesterol concentration seen with consumption of cafestol (1). Evidence from kinetic studies indicates that only a small part of $VLDL_1$ is delipidated to LDL (42, 43), whereas $VLDL_2$ particles are rapidly and efficiently converted to LDL (42). Therefore, cholesteryl ester–enriched $VLDL_2$ particles appear to be the more likely candidate to play a role in the subsequent rise in LDL-cholesterol concentrations with consumption of unfiltered coffee (1). In addition, in vitro studies suggest that cafestol elevates plasma LDL-cholesterol concentrations at least partly by down-regulation of the LDL receptor (44–46). This finding was recently confirmed in vivo in apo E*3-Leiden transgenic mice (41). Down-regulation of the LDL receptor appears to be initiated by suppression of bile acid synthesis (41, 46). The action of cafestol on plasma triacylglycerol and plasma cholesterol might thus be regulated independently in the liver.

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