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Absorption and urinary excretion of the coffee diterpenes cafestol and kahweol in healthy ileostomy volunteers

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Abstract. de Roos B, Meyboom S, Kosmeijer-Schuil TG, Katan MB (Wageningen Agricultural University, Wageningen, The Netherlands). Absorption and urinary excretion of the coffee diterpenes cafestol and kahweol in healthy ileostomy volunteers. *J Intern Med* 1998; **244:** 451–60.

Objectives. To determine the absorption and urinary excretion of the cholesterol-raising coffee diterpenes cafestol and kahweol in humans.

Subjects and design. Nine healthy ileostomists consumed a dose of one, two or three cups of French-press coffee together with a standardized breakfast on three separate days in random order. Subsequently, ileostomy effluent was collected for 14 h and urine for 24 h. Stability of cafestol and kahweol was also assessed under simulated gastrointestinal tract conditions.

Main outcome measures. Absorption of diterpenes, stability of diterpenes during incubation with gastrointestinal fluids, and urinary excretion of diterpenes.

Results. Corrected mean absorptions expressed as percentages of the amount consumed and the amount entering the duodenum were 67 and 88%, respectively, for cafestol, and 72 and 93%, respectively, for kahweol. We found losses of diterpenes during incubation in vitro with gastric juice (cafestol, 24%; kahweol, 32%), during storage with ileostomy effluent (cafestol, 18%; kahweol, 12%), and during freeze-drying (cafestol, 26%; kahweol, 32%). Mean excretion of glucuronidated plus sulphated conjugates in urine was 1.2% of the ingested amount for cafestol and 0.4% of the ingested amount for kahweol.

Conclusions. About 70% of the ingested cafestol and kahweol is absorbed in ileostomy volunteers. Possibly, undetected metabolites are present in ileostomy effluent, resulting in lower absorption percentages. Only a small part of the diterpenes is excreted as a conjugate of glucuronic acid or sulphate in urine. Therefore, these compounds are extensively metabolized in the human body.

Keywords: absorption, coffee diterpenes, ileostomy volunteers, serum lipids, urinary excretion.

Introduction

Cafestol and kahweol (Fig. 1) are naturally occurring diterpenes in coffee beans [1], where they are present as fatty esters, mainly of palmitic and linoleic acids [2]. They are responsible for the raising of low density lipoproteins (LDL) and very low density lipoproteins (VLDL) brought about by drinking Scandinavian-type boiled coffee [3,4], which contains 3–4 mg of each per cup [1]. Levels of diterpenes are also high in other unfiltered coffee brews such as Turkish/Greek and French-press or cafetiere coffee [1]. The LDL- and VLDL-raising effect of cafestol and kahweol seems to be unique in humans: serum lipids in various animal models did not respond to the two diterpenes [5].

In humans, cafestol appears to be mainly responsible for the effects on serum lipids [5, 6]. A meta-analysis on 11 trials showed that a daily dose of 10 mg of cafestol for 4 weeks raised serum cholesterol by 0.13 mmol L^{-1}, whilst kahweol raised serum cholesterol by 0.02 mmol L^{-1}. About 80% of the rise in serum cholesterol was accounted for by LDL cholesterol. A daily dose of 10 mg of cafestol or kahweol for 4–6 weeks raised serum triglycerides by 0.08 and 0.01 mmol L^{-1}, respectively. Most of the rise in serum triglycerides subsides with chronic intake of coffee diterpenes [5]. Both cafestol and kahweol also appear

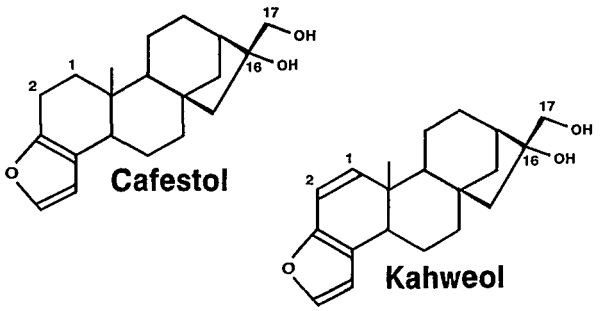

Fig. 1 Structure of the coffee diterpenes cafestol and kahweol. Diterpenes occur in coffee beans mainly esterified to fatty acids at the C16 or C17 position.

to affect liver cell functioning in humans: each 10 mg day^{-1} of cafestol or kahweol increased alanine aminotransferase by 2–3 U L^{-1} after 4 weeks [5]. The different capacities of cafestol and kahweol to raise serum lipids or serum liver enzymes indicate that possibly two distinct mechanisms of action are involved.

Knowledge about the absorption and the mechanisms of action of these coffee diterpenes in humans is limited. *In vitro* studies suggest that the coffee diterpenes decrease receptor-mediated uptake of LDL cholesterol [7, 8]. A lower hepatic uptake of LDL cholesterol may contribute to the cholesterol-raising effect of cafestol observed in humans. Absorption values are now required to assess which part of the consumed diterpenes is actually responsible for the rise in serum lipids. Factors such as dose might influence absorption, and thus the effects observed. In addition, absorption values indicate which part of the consumed diterpenes are unabsorbed and thus enter the colon, where they might have beneficial effects; intake of coffee diterpenes reduced the frequency of adenocarcinoma of the colon in rats [9], and in some human epidemiological studies a lower incidence of colon cancer with coffee intake was found [10]. More insight into the metabolism of cafestol and kahweol in the human body might lead us to the compound which actually raises serum lipids. This active compound could be cafestol or kahweol, but it could also be a metabolite. Such insights would facilitate further studies of the mechanism of action.

We assessed the absorption of cafestol and kahweol from increasing doses of French-press coffee by measuring coffee diterpenes in ileostomy effluent of healthy ileostomy volunteers. We also provide evidence for the excretion of conjugated cafestol and kahweol metabolites in urine.

Subjects and methods

We obtained prior approval for the experiment from the medical ethical committee of the Department of Human Nutrition, Wageningen University.

Subjects

We recruited subjects by writing to members of the Dutch association of ileostomists and by approaching volunteers from a previous study [11]. Thirteen subjects filled out a general and a medical questionnaire and blood samples were taken for standard laboratory assays. Exclusion criteria were as follows: signs of Crohn's disease or malabsorption; resection of more than 50 cm of the terminal ileum; an ileostomy that did not function properly; a history of hepatobiliary disease, renal disease or diabetes; use of drugs influencing gastrointestinal transit; present illness; pregnancy or lactation; a serum haemoglobin concentration of less than 8.6 mmol L^{-1} for men or less than 7.4 mmol L^{-1} for women; serum cholesterol levels of more than 8.0 mmol L^{-1}; triglyceride levels of more than 2.37 mmol L^{-1}; or ALT levels exceeding the upper limit of normal. We checked urine samples for protein and glucose. The medical questionnaires and the results of the blood tests were judged by an independent gastroenterologist. Four subjects were excluded from this study because of Crohn's disease (n = 1), liver and gall bladder disease (n = 1), small intestine disease (n = 1) or removal of more than 50 cm of the terminal ileum (n = 1).

Nine subjects (four men and five women) with a mean age of 60 years (range 43–71 years) and mean (± SD) body mass index of 26.8 ± 3.0 kg m^{-2} were admitted to the study. All subjects had undergone proctocolectomy 3–27 years earlier because of ulcerative colitis (n = 8) or polyposis coli (n = 1), and all had an intact ileum. The subjects gave their informed consent before the start of the study.

Supplements

We prepared French-press coffee by pouring 1 L of boiling water onto 65 g of coarsely ground coffee (Roodmerk, Douwe Egberts, The Netherlands) in a glass jug (Kaffee Primo, BMF, Germany). The brew was thoroughly stirred and allowed to stand for

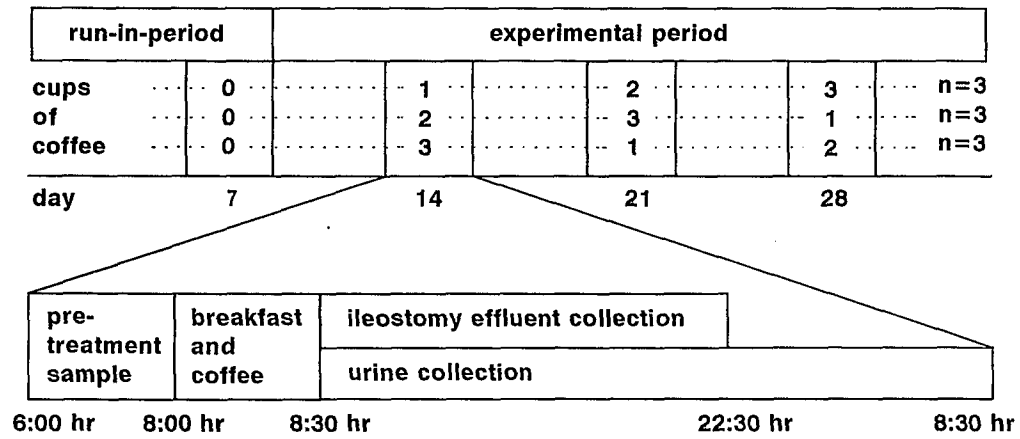

Fig. 2 Design of the study. Nine healthy ileostomy volunteers followed a run-in period of 7 days and were randomly assigned to three treatment sequences, each consisting of three treatment days. On day 7, a standardized breakfast was provided and subsequently ileostomy effluent was collected every 2 h for about 14 h. On days 14, 21 and 28, a standardized breakfast with a single dose of one, two or three cups of French-press coffee was provided, ileostomy effluent was collected every 2 h for about 14 h, and urine was collected for 24 h. On the day when three cups of French-press coffee were consumed, subjects collected urine every 2 h. During the night preceding every experimental day, pretreatment samples of ileostomy effluent and urine were obtained.

3 min. The plastic strainer was pushed down and the liquid was decanted. For each subject, we weighed 150, 300 or 450 mL of the coffee brew into a thermos flask so as to provide one, two or three cups of coffee, respectively. We stored another 100 mL of each individual brew at −20 °C for diterpene analysis.

Study design

We used a multiple cross-over design (Fig. 2) in which all volunteers first followed a run-in period of 7 days. On day 7, subjects received a standardized breakfast without coffee. Subsequently, they were randomly assigned to three treatment sequences, each consisting of three treatment days separated by one washout week. Treatment on days 14, 21 and 28 consisted of consumption of the same standardized breakfast as on day 7, plus one, two or three cups of French-press coffee. Subjects also swallowed 20 radio-opaque barium-salt-impregnated plastic ringlets with an outer diameter of 3 mm (TD Medical, Eindhoven, The Netherlands). These markers provide a validated method for the assessment of solid food recovery in ileostomists [12]. Subjects were restricted from eating and drinking for 2 h after breakfast, except for having tea without milk or sugar, or water. On all treatment days, subjects swallowed with their meals three capsules containing 80 mg day^{-1} of *para*-aminobenzoic acid each to check for complete urine collection. *Para*-aminobenzoic acid is completely absorbed and excreted with urine in humans [13]. The participants were allowed to drink only filtered or instant coffee throughout the study, as these coffee types contain negligible amounts of cafestol and kahweol [1]. Participants recorded any deviation from their usual diet and physical activity pattern in a diary, as well as any signs of illness and medication used.

Collection of samples

On days 7, 14, 21 and 28, subjects collected ileostomy effluent throughout the day for 14.1 ± 0.7 h (mean ± SD) after coffee consumption. They also collected a pretreatment sample during the night preceding each experimental day. Subjects changed ileostomy bags every 2.1 ± 0.2 h and immediately stored them on dry ice. We counted the number of radio-opaque ringlets in the ileostomy samples by X-raying within the next 2 days. We separated the plastic ileostomy bags from their contents using liquid nitrogen, and freeze-dried the contents. We checked sample weights once or twice a day during the process of freeze-drying and immediately removed them from the freeze-dryer when stable weight was reached. Samples were ground to pass a 0.5 mm sieve and stored at −20 °C until analysis.

Subjects collected urine for 24 h after each coffee dose. Control urine samples were collected during the night preceding each treatment day. On the day on which three cups of French-press coffee were provid-

ed, subjects collected 24-h urine in timed portions every 2.0 ± 0.1 h during the daytime and in one portion during the night. Urine was voided into 0.5 L nalgene bottles containing 5 mL toluene (Janssen Chimica) each, and immediately put on dry ice. The following morning we warmed the urine samples to 37 °C, mixed and took aliquots for cafestol, kahweol and *para*-aminobenzoic acid analysis. Aliquots were stored at −20 °C.

In vitro *incubation of diterpenes with gastrointestinal fluids*

We evaluated the stability of cafestol and kahweol palmitate in gastric and duodenal juice *in vitro* in order to asses possible losses of diterpenes during gastrointestinal transit. Gastric and duodenal juice were obtained from two healthy fasting volunteers at the Department of Gastroenterology, Nijmegen University Hospital, The Netherlands, and stored at −20 °C. We incubated 6, 12 and 18 mL of French-press coffee with 4 mL of gastric juice and 1 mL of water each for 0, 20 and 60 min at 37 °C. This mimicked stomach contents after the administration of one, two and three cups of coffee [14, 15]. To assess whether a low pH affects the recovery of diterpenes during stomach passage, we made five solutions of HCl in demineralized water with pH values of 0.72, 1.43, 2.19, 3.56 and 4.05. One volume of each solution was added to three volumes of French-press coffee. This yielded incubation samples with pH values of 1.56, 3.89, 4.63, 4.81 and 4.92, respectively. Distilled water was used for the control incubation. Samples were incubated at 37 °C for 20 and 60 min, frozen immediately, and stored at −20 °C. In addition, we incubated 6, 12 and 18 mL of French-press coffee with 2 mL of duodenal juice (Department of Gastroenterology, Nijmegen University Hospital, The Netherlands) plus 1 mL of water each for 0, 1 and 4 h; 1 and 4 h reflect the estimated mean and maximum transit time in the human small intestine, respectively [16, 17]. All samples were frozen with liquid nitrogen immediately after the incubation, and stored at −20 °C.

We also assessed stability of cafestol and kahweol palmitate during collection of the effluent in the ileostomy bag and during freeze-drying. To that end, we incubated 70 mg of a mixture of cafestol plus kahweol palmitate dissolved in 0.5 mL ethanol with 50 g of fresh ileostomy effluent obtained from 8 subjects for 2 and 0 h, respectively, at 30 °C. The samples were shaken every 15 min during incubation. Samples were immediately frozen in liquid nitrogen, freeze-dried and stored at −20 °C. To assess the stability of cafestol and kahweol during freeze-drying in detail, we dissolved 40.7 mg aliquots of cafestol plus kahweol palmitate in 0.4 mL ethanol plus 9.6 mL distilled water, and freeze-dried such samples for various time periods.

Analytical methods

Cafestol and kahweol in ileostomy effluent. We analysed samples with and without pretreatment with an enzyme preparation having β-glucuronidase activity (100 000 units mL^{-1}) and sulphatase activity (5000 units mL^{-1}) (Sigma, G7017). The pretreatment consisted of incubating 300 mg of thawed ileostomy effluent with 200 μL of β-glucuronidase and 2 mL of 0.4 mol L^{-1} phosphate buffer (pH 5) in a water bath of 37 °C for 1 h. Subsequently, we prepared samples for diterpene analysis as described by Urgert *et al.* [1]. Briefly, we added 2 mL of 5 mol L^{-1} methanolic KOH to both pretreated and untreated samples, and saponified the samples for 1 h at 80 °C. After cooling the samples, lipids were extracted by adding 5 mL of diisopropyl ether (Merck, Darmstadt, Germany, no. 867), shaking for 10 min at 250 oscillations min^{-1} and centrifuging for 10 min at 1580 ***g***. We collected the ether phase and re-extracted the water phase twice with diisopropyl ether. The combined ether phases were washed twice with 3 mL of distilled water. The ether phase was evaporated with nitrogen, and the remnant was dissolved in 1 mL of methanol (Lab Scan, no. C2517). Of each sample, 10 μL was injected into an HPLC system (Shodex degas, SP8875 autosample, SP8800 ternary HPLC pump, Spectra Focus detector, Thermo Separation Products) equipped with two serially connected reverse-phase Spherisorb ODS columns, 100 × 3 mm each (Chrompack, Middelburg, The Netherlands). The elution solvent, which consisted of 62.5% (v/v) methanol and 37.5% filtered distilled water, was administered at a flow rate of 0.4 mL min^{-1}. Detection was at both 220 and 290 nm, which reflects the optimal absorption of cafestol and kahweol, respectively. The coefficients of variation for a control sample of faeces containing a known amount of cafestol and kahweol were 10.6% within, and 7.0% between, runs over a 6-month

period for cafestol, and 8.2 and 7.1%, respectively, for kahweol.

Cafestol and kahweol in urine. The urine samples were thawed and centrifuged. Two millilitres of the supernatant was incubated with 1 mL of 0.4 mol L–1 phosphate buffer (pH 5) and 200 μL of β-glucuronidase at 37 °C for 1 h, adsorbed to a 500 mg reverse-phase C18 column (Varian products, USA), washed twice with 1 mL of distilled water, and extracted with 3 mL of methanol. The extract was evaporated and the remnant dissolved in 250 μL methanol/H2O, 62.5/37.5 v/v. Twenty microlitres was injected into the HPLC system, equipped with two serially connected reverse-phase ChromSpher C8 columns, 100 × 3 mm each (Chrompack). Other conditions were as described for ileostomy effluent. Two urine samples from a previous experiment [18], one with a low and one with a high cafestol and kahweol content, showed, respectively, coefficients of variation for cafestol of 14.4 and 4.6% within runs, and 15.8 and 9.8% between runs over a 6-month period. For kahweol, coefficients of variation were, respectively, 25.8 and 11.1% within runs and 12.8% and 16.1% between runs.

Cafestol and kahweol in coffee and gastrointestinal fluids. We analysed coffee and fluid samples as described by Urgert *et al.* [1]. The coefficients of variation for a control pool of boiled coffee were 2.5% within and 9.5% between runs over a 6-month period for cafestol, and 2.3 and 7.2%, respectively, for kahweol.

Para-*aminobenzoic acid in urine.* We determined *para*-aminobenzoic acid spectrophotometrically with fluorescamine (Roche, 071088) as described previously [19]. Addition of 200 μg of *para*-aminobenzoic acid to 1 mL of urine yielded a recovery of 96% ($n = 2$).

Results

Collection of ileostomy effluent and urine

Of the 20 radio-opaque ringlets swallowed, 93 ± 13% (mean ± SD) were recovered in the ileostomy bags. One subject refrained from swallowing the ringlets on the third treatment day after consultation with the researchers, because during the first two experimental days she had excreted less than 50% of the 20 ingested radio-opaque ringlets. Data analysis was performed with and without this subject's results on diterpene excretion in ileostomy effluent.

Recovery of *para*-aminobenzoic acid in 24-h urine was 85 ± 16% (mean ± SD). Four persons showed, on 10 separate occasions, an urinary recovery of *para*-aminobenzoic acid of less than 85%. Data analysis was performed with and without the results of these 10 occasions.

Stability of cafestol and kahweol during incubation with gastrointestinal fluids

During *in vitro* incubation in gastric juice, recoveries of cafestol decreased by 25 ± 10% (mean ± SD, $n = 6$ incubations) after 20 min and 23 ± 9% after 60 min. Recovery of kahweol decreased by 22 ± 9% ($n = 6$ incubations) after 20 min and 25 ± 6% after 60 min. Outcomes were irrespective of the dose of French-press coffee, and therefore data were pooled per incubation time period. Recoveries of cafestol and kahweol after incubation with HCl dillutions with pH values of 0.7, 1.4, 2.2, 3.6 and 4.1 were 89 ± 9 (mean ± SD, $n = 8$ incubations), 112 ± 3, 113 ± 6, 116 ± 5 and 113 ± 6%, respectively. We pooled data per pH because outcomes were irrespective of the incubation time. The diterpenes were stable during incubation in duodenal juice for 1 and 4 h. Incubation of cafestol and kahweol with ileostomy effluent for 2 h plus subsequent freeze-drying yielded a recovery of 56 ± 5% ($n = 6$ incubations) for both cafestol and kahweol.

Stability of cafestol and kahweol during freeze-drying

Recovery of cafestol and kahweol palmitate in ileostomy effluent during freeze-drying was 74 ± 5% ($n = 4$ incubations) for cafestol and 68 ± 4% ($n = 4$ incubations) for kahweol. Cafestol plus kahweol palmitate dissolved in ethanol and distilled water were stable during freeze-drying until all water had disappeared, i.e. after 8 h. Then recovery decreased to 60% for cafestol and 37% for kahweol during an additional 22 h of freeze-drying. Therefore, loss of cafestol and kahweol during freeze-drying appears to occur mainly after samples have lost their water content.

Calculations

We used an algorithm to correct the absorption of cafestol and kahweol in the small intestine for losses

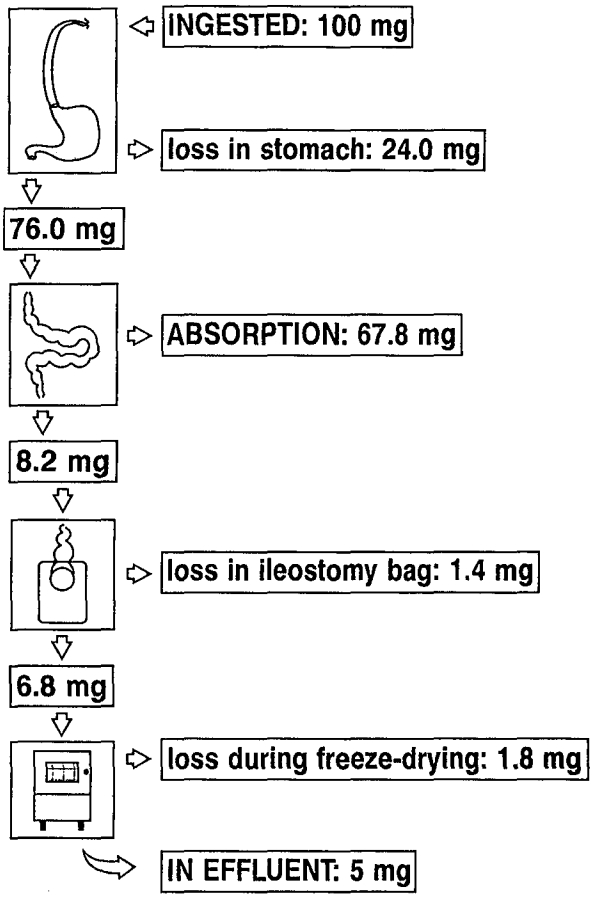

Fig. 3 Algorithm to calculate absorption of cafestol and kahweol. Data were calculated as described in the 'Results' section. Losses during incubation with gastric juice, during storage in ileostomy effluent and during freeze-drying were estimated with data obtained from *in vitro* experiments.

in gastric juice, in ileostomy effluent and during freeze-drying (Fig. 3). We calculated the individual absorption of cafestol and kahweol, expressed as a percentage of the amount consumed, as follows:

cafestol absorption as a percentage of the amount consumed

$$(\%) = (\{(X - 0.24X) - [(Y/0.74)/0.83]\}/X) \times 100$$

kahweol absorption as a percentage of amount consumed

$$(\%) = (\{(X - 0.23X) - [(Y/0.68)/0.88]\}/X) \times 100$$

where X was the amount consumed with the French-press coffee, and Y the amount detected in freeze-dried ileostomy effluent. Absorption of cafestol and kahweol, expressed as a percentage of the amount entering the duodenum, was calculated as follows:

cafestol absorption as a percentage of the amount entering the duodenum

$$(\%) = (\{(X - 0.24X) - [(Y/0.74)/0.83]\}/(X - 0.24X)) \times 100$$

kahweol absorption as a percentage of the amount entering the duodenum

$$(\%) = (\{(X - 0.23X) - [(Y/0.68)/0.88]\}/(X - 0.23X)) \times 100$$

Cafestol and kahweol excretion in urine were corrected for estimated losses in the stomach (cafestol, 24%; kahweol, 23%).

Absorption and excretion of coffee diterpenes

No cafestol or kahweol was found in ileostomy effluent collected on the pre-experimental control day (Fig. 2, day 7), when no French-press coffee was provided. However, significant amounts of the coffee diterpenes were found in pretreatment samples of two subjects collected in the night preceding an experimental day. Data analyses were therefore performed with and without the results obtained from the subsequent two experimental days. The reason for the presence of the coffee diterpenes in the pretreatment samples is not clear. Both subjects declared not to have consumed any coffee other than filtered coffee during the experiment. It might be that cafestol and kahweol are not unique in coffee beans. So far, however, these coffee diterpenes have not been found in other foods.

Only free cafestol and kahweol was detected in ileostomy effluent; addition of β-glucuronidase with sulphatase activity did not result in a higher diterpene content (data not shown). Mean (± SD) absorptions of cafestol and kahweol, expressed as percentages of the ingested amount and the amount entering the duodenum, after correction for the estimated losses, are shown in Table 1.

No free cafestol or kahweol was found in urine. Urinary excretion of glucuronidated or sulphated cafestol and kahweol occurred mostly within 8 h after consumption of the French-press coffee, indicating that collection was complete. There was a considerable variation in the subjects' excretion rates, possibly due to interindividual differences in phase II enzyme activities (Fig. 4). Mean (± SD) excretion of cafestol and kahweol in 24-h urine is illustrated in Table 1.

Table 1 Intake, excretion and estimated absorption of cafestol and kahweol from French-press coffee expressed as percentages of the ingested amount and the amount entering the duodenum in nine healthy ileostomy volunteers.* Subjects consumed, on three separate days, a standardized breakfast with one, two or three cups of French-press coffee. They subsequently collected ileostomy effluent for 14 h and urine for 24 h

Diterpene and cups of coffee	*n*	Intake (mg)	Excretion in ileostomy effluent (mg)	Absorption of ingested amount† (%)	Absorption of amount entering the duodenum† (%)	Excretion in 24-h urine‡	
						(mg)	(%)
Cafestol							
1 cup	9	6.1 ± 1.3 (4.6–7.6)	0.4 ± 0.4 (0.1–1.0)	64.2 ± 10.2	84.5 ± 13.5	0.1 ± 0.1 (0.0–0.4)	1.8 ± 4.1
2 cups	9	12.9 ± 2.6 (9.3–16.1)	0.8 ± 0.6 (0.2–2.0)	65.8 ± 7.7	86.6 ± 10.1	0.1 ± 0.1 (0.0–0.1)	0.4 ± 0.4
3 cups	9	20.9 ± 4.8 (12.7–24.3)	0.7 ± 0.6 (0.1–1.6)	70.6 ± 3.9	92.9 ± 5.1	0.2 ± 0.2 (0.0–0.5)	1.4 ± 0.7
Kahweol							
1 cup	9	6.8 ± 1.4 (5.2–8.2)	0.3 ± 0.3 (0.0–0.8)	70.2 ± 7.6	91.1 ± 9.8	0.0 ± 0.0 (0.0–0.1)	0.5 ± 1.1
2 cups	9	14.4 ± 2.7 (10.8–17.5)	0.4 ± 0.4 (0.1–1.4)	7.2 ± 4.5	93.9 ± 5.8	0.0 ± 0.0 (0.0–0.1)	0.2 ± 0.2
3 cups	9	23.0 ± 4.7 (14.7–26.3)	0.5 ± 0.5 (0.1–1.7)	73.2 ± 3.0	95.0 ± 3.9	0.1 ± 0.1 (0.0–0.2)	0.5 ± 0.4

*Mean ± SD (range). Analysis was performed on all subjects. Mean absorption of cafestol expressed as a percentage of the amount consumed – after rejection of the results on two occasions where cafestol and kahweol were found in pretreatment samples and of the results of one subject who refrained from swallowing the radio-opaque ringlets – were 66.7% (n = 7), 65.4% (n = 8) and 70.8% (n = 7) after consumption of one, two or three cups of French-press coffee, respectively. Mean absorption of kahweol was 72.0, 72.1 and 72.7%, respectively. Cafestol absorption expressed as a percentage of the amount entering the duodenum was 87.8, 86.1 and 93.1% after consumption of one, two or three cups of French-press coffee, respectively. Mean absorption of kahweol was 93.5, 93.6 and 94.4%, respectively. Mean excretion of cafestol in 24-h urine after exclusion of the results of the two occasions were diterpenes were found in pretreatment samples and of the results of the occasions where less than 85% of *para*-aminobenzoic acid was recovered in urine was 0.0% (n = 5), 0.2% (n = 5) and 0.9% (n = 6) after consumption of one, two or three cups of French-press coffee, respectively. Mean excretion of kahweol was 0.1, 0.1 and 0.3%, respectively.

†Corrected for estimated loss in gastric juice (cafestol, 24%; kahweol, 23%), estimated loss in the ileostomy bag (cafestol, 18%; kahweol, 12%) and estimated loss during freeze-drying (cafestol, 26%; kahweol, 32%) and calculated as described in the 'Results' section.

‡Corrected for estimated loss in gastric juice (cafestol, 24%; kahweol, 23%).

Discussion

We found that about 70% of the ingested cafestol and kahweol from French-press coffee was absorbed in ileostomy volunteers. Most of the other 30% was degraded in gastric fluid before it could reach the duodenum. Absorption of the cafestol and kahweol that reached the duodenum was about 90%. Only a small part was subsequently excreted as a conjugate of glucuronic acid or sulphate in urine.

Validity of the ileostomy model

We studied the excretion of cafestol and kahweol in subjects whose colon had been resected. The advantage of the ileostomy model is the absence of microbial degradation in the colon. When 2-h portions of ileostomy effluent during daytime and one portion during the night were subjected to immediate freezing on dry ice, virtually no microbial degradation on protein, fat and dietary fibre in the ileostomy bags was found [20, 21], and degradation of bile acids and neutral sterols was absent or minimal [22, 23]. In our samples, however, we did find a mean degradation of 18% of cafestol and 12% of kahweol 2 h after addition to freshly collected ileostomy effluent. A part of the cafestol and kahweol reaching the ileostomy bag might be degraded by microflora excreted from the terminal ileum. Substantial bacterial colonization of the terminal ileum in ileostomists has been reported [24–26]. If indeed bacteria are responsible for the degradation of cafestol and kahweol in ileostomy effluent, use of the ileostomy model does not appear to exclude effects of microflora on all substances present in the ileostomy bag.

We could not avoid freeze-drying, since this is the only way to obtain homogeneous samples for the analysis. Losses of cafestol and kahweol during freeze-drying might be due to the formation and sub-

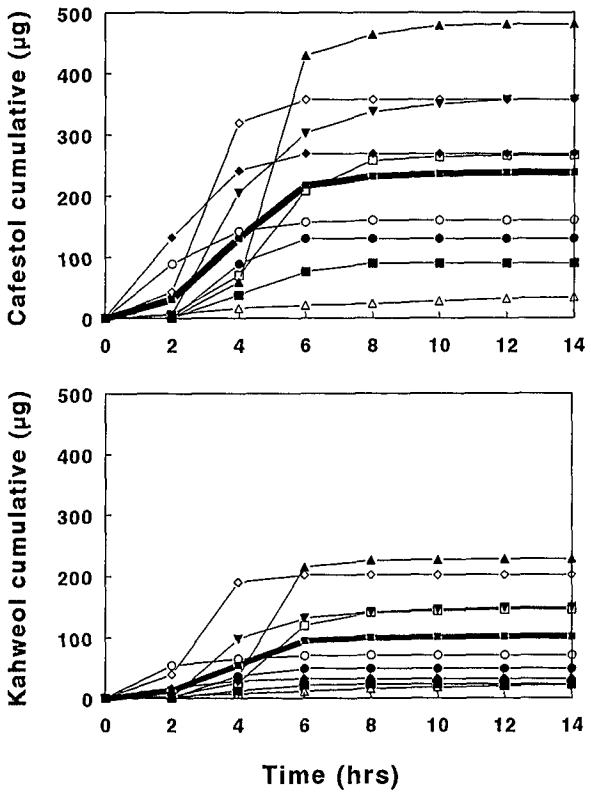

Fig. 4 Cumulative excretion of cafestol and kahweol in the urine of nine subjects after consumption of three cups of French-press coffee. The heavy line represents the mean.

limation of cafestolene and kahweolene; these contain an additional double bond between C15 and C16 compared with the original diterpenes [27]. Loss of cafestol and kahweol became high after the water had disappeared. However, we immediately removed samples from the freeze-dryer upon reaching stable weight. Therefore, losses of cafestol and kahweol were minimized.

Gastrointestinal transit of coffee diterpenes

We found a considerable loss of cafestol and kahweol present in French-press coffee during *in vitro* incubation with representative amounts of gastric juice. Although the number of *in vitro* incubations were few, the results were consistent. Therefore, these *in vitro* data strongly indicate that at least a part of the consumed diterpenes are transformed into other compounds during gastric passage. As the recoveries of cafestol and kahweol with gastric juice were equally affected, the double bond between C1 and C2 in the kahweol molecule does not appear to play an important role. Recovery of cafestol and kahweol was slightly smaller after incubation with a solution with a pH value of 0.72, compared with solutions with pH values ranging from 1.43 to 4.05. Therefore, high concentrations of HCl might be responsible, in part, for the transformation of diterpenes during incubation with gastric juice. However, the 25% decrease in recovery of coffee diterpenes during incubation with gastric juice cannot be fully explained by low pH.

Absorption of coffee diterpenes

An algorithm based on losses determined *in vitro* enabled us to estimate the absorption of cafestol and kahweol, expressed as a percentage of the amount consumed and of the amount entering the duodenum. Both calculated mean absorptions might show variation between the nine subjects because of interindividual differences in the proportion of diterpenes in ileostomy effluent compared with the amount consumed. Our algorithm, however, does not consider the variation of the mean losses determined *in vitro* in the calculation of individual absorption values. Therefore, the real variation in mean absorption will be underestimated. We calculated that the standard deviation of the amounts of cafestol and kahweol leaving the stomach (in the formula presented as $X - 0.24X$ for cafestol and $X - 0.23X$ for kahweol) has 10 times more influence on the total variation of an individual absorption value than the standard deviation of the amount entering the colon (in the formula presented as $(Y/0.74)/0.83$ for cafestol and $(Y/0.68)/0.88$ for kahweol). These standard deviations taken together lead to coefficients of variation of 16% for the absorption of cafestol expressed as a percentage of the ingested amount, and 13% for the absorption of kahweol as a percentage of the ingested amount. This indicates that two-thirds of the absorption values expressed as a percentage of the ingested amount will lie in a range of 52–84% for cafestol and 56–81% for kahweol.

As estimated losses were about equal for both cafestol and kahweol, excretion in ileostomy effluent is expected to be similar. However, kahweol excretion was somewhat lower than that of cafestol. This is in agreement with earlier observations in faeces [28]. Kahweol might be absorbed more efficiently than cafestol. Also, the double bond of the kahweol mole-

cule might be hydrogenated by bacteria present in the terminal ileum.

Collection of ileostomy effluent during the daytime appeared to cover all cafestol and kahweol excreted. Pilot experiments revealed that no cafestol or kahweol was present in ileostomy effluent sampled 12 h after the consumption of French-press coffee (data not shown).

Urinary excretion of coffee diterpenes

We measured excretion of diterpenes as a conjugate of glucuronic acid and sulphate in urine, because glucuronidation and sulphation are the major pathways of xenobiotic biotransformation in mammalian species [29]. We hypothesized that at least a part of the ingested cafestol and kahweol will not undergo phase I metabolization, as they already possess hydroxyl groups. Conjugation of this part with a hydrophilic moiety appears to be necessary for excretion of the fat-soluble coffee diterpenes by the kidney. Indeed, no free cafestol or kahweol was present in urine from subjects receiving diterpene-rich supplements in previous experiments [28]. However, since only about 1% of the ingested amount was excreted as conjugate of glucuronic acid or sulphate in urine, the major part of the absorbed diterpenes must be metabolized more extensively than just glucuronidation or sulphation of the cafestol and kahweol molecules.

There are some indications that phase I metabolites might undergo conjugation with glutathione; kahweol palmitate and, to a lesser extent, cafestol palmitate induced glutathione-*S*-transferases activity in the mucosa of the small intestine and in the liver of mice [30]. The furan moiety of cafestol and kahweol appears vital for this effect [31].

We did not find glucuronidated or sulphated conjugates of cafestol and kahweol in ileostomy effluent. This implies that cafestol and kahweol conjugates of glucuronic acid or sulphate are small enough to be excreted into urine instead of into bile, or that the ileostomy effluent matrix inhibits β-glucuronidase. Also, the fact that we found only a small percentage of the absorbed cafestol and kahweol as conjugates of glucuronic acid or sulphate in urine suggests that glucuronidation and sulphation of cafestol and kahweol are minor processes. Since we did not measure metabolites of cafestol and kahweol other than conjugates of glucuronic acid and sulphate, the presence of other metabolites might overestimate the absorption percentages as calculated with the algorithm.

In conclusion, about 90% of the cafestol and kahweol that enters the small intestine is absorbed there. Absorption of these coffee diterpenes expressed as a percentage of the amount consumed is about 70%. This indicates that of each 10 mg of cafestol in French-press coffee consumed, 7 mg of cafestol is absorbed in the small intestines and available for raising serum cholesterol by about 0.13 mmol L^{-1} in humans. The question remains as to which part of the ingested diterpenes eventually raises serum lipids, since we did not study the fate of cafestol and kahweol in the pre-systemic circulation. Only 0.8 mg will enter the colon; the amounts available for the presumed anticarcinogenic effects of coffee diterpenes [10] are thus very small. Moreover, only a very small amount of the cafestol and kahweol which enters the circulation is subsequently excreted as conjugate of glucuronic acid or sulphate in urine. Therefore, the major part of the ingested cafestol and kahweol must be metabolized differently from just glucuronidation or sulphation of the cafestol and kahweol molecules.

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