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Intake levels, sites of action and excretion routes of the cholesterol-elevating diterpenes from coffee beans in humans

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Introduction

Europeans use on average 4.6 kg of green coffee beans per year, which is roughly equivalent to two cups of coffee per day. Scandinavian countries and the Netherlands are highest, at over 10 kg/year, while U.K. citizens are moderate coffee drinkers with an average of 2.5 kg/year [1].

Coffee is mostly consumed for its caffeine content. Caffeine is responsible for the stimulating effect of coffee, possibly through inhibition of brain adenosine receptors. Caffeine exerts a range of physiological activities, but appears to have little adverse effect on health (for a review see [2]).

Two other active compounds from coffee beans are the lipid-soluble diterpenes *cafestol* and *kahweol*. They have recently been shown to be responsible for the cholesterol-raising effect of Scandinavian boiled coffee [3,4]. We here describe the identification of these cholesterol-raising factors from coffee beans, and address their physiological effects in humans. We also provide new data on excretion routes of cafestol and kahweol.

Abbreviations used: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyltransferase; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein.

The cholesterol-elevating factor from coffee beans

The relation between coffee drinking and risk factors for coronary heart disease have long been controversial. In 1983, a strong association between coffee drinking and the serum concentration of cholesterol was reported from Norway [5]. However, this relationship was highly inconsistent in the U.S.A. and in Western European countries (for a review see [6]). It soon turned out that this was due to differences in brewing method: Norwegians traditionally boil coffee grounds with water, and consume the brew without filtering, while Americans and Western Europeans mostly use paper-filtered coffee. In experiments with volunteers it was shown that

'boiled' coffee markedly increases serum cholesterol levels [7,8], and that it loses its cholesterol-raising potency when it is poured through a paper filter [9,10].

The epidemiological data now became unambiguous: serum cholesterol levels rose with the intake of boiled coffee, but much less or not at all with the intake of paper-filtered coffee [11–14]. In Finland, about 75% of the coffee drinkers have switched from boiled to filtered coffee during the last 25 years, and this is thought to explain about one-third [6] to one-half [15] of the 10% reduction in mean serum cholesterol levels of Finnish people over the same time period.

A key observation in explaining the effect of brewing technique was that boiled coffee contains 1–2 g of oil/l, whereas the lipid content of filtered coffee is negligible [9,16]. Ingestion of 1.3 g of lipids isolated from unfiltered coffee per day raised serum cholesterol levels by 23% in volunteers [17]. About 70% of this rise was accounted for by an increase in cholesterol carried by low-density lipoprotein (LDL) particles. However, there was also a sharp rise in fasting triacylglycerols or very-low-density lipoprotein (VLDL) particles, and a slight dip in the antiatherogenic high-density lipoprotein particles.

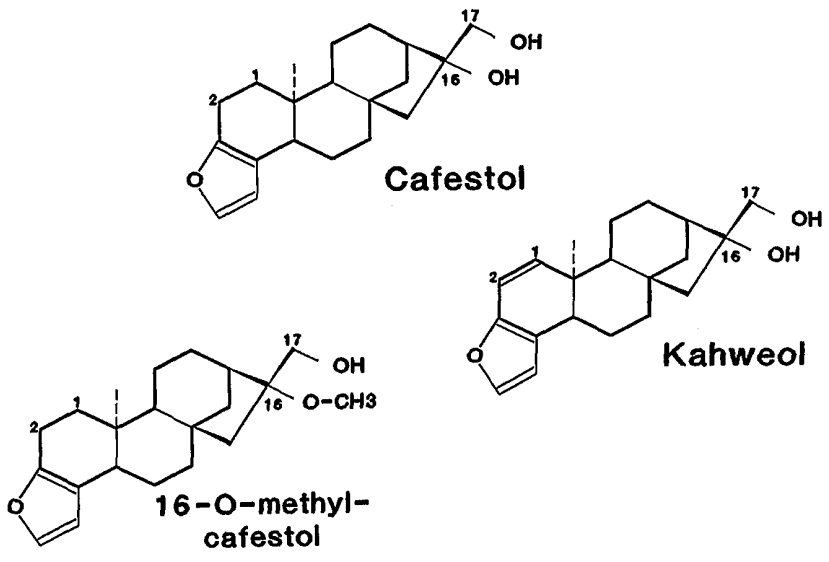
Coffee lipids consist mainly of triacylglycerols, but 10–15% are diterpene alcohols esterified to fatty acids [16,18]. We found that coffee oil that had been stripped of these diterpenes had lost its LDL- and VLDL-raising potential [3]. Later experiments confirmed that purified coffee diterpenes are strongly hyperlipidaemic in humans [3,4,19].

The main diterpenes in coffee are cafestol and kahweol. They differ in that kahweol has a double bond between the C-1 and C-2 carbon atoms (Figure 1). In a cross-over study with ten healthy volunteers, we found that daily consumption of 60 mg of cafestol alone significantly elevated serum levels of cholesterol by 15% and those of fasting triacylglycerols by 84%. Addition of 50 mg of kahweol to the cafestol treatment produced only modest further increases in blood lipid levels; serum levels of cholesterol were elevated by 20% and those of triacylglycerols by 99% [19]. The C-1–C-2 double bond of kahweol evidently impairs its capacity to affect lipid metabolism in humans, and cafestol appears to be the principal hyperlipidaemic factor from coffee beans. From our experiments, we estimated that each 10 mg of cafestol ingested per day raises serum cholesterol levels by 0.13 mmol/l [3].

Figure 1

Structure of the coffee diterpene alcohols cafestol, kahweol and 16-O-methylcafestol

Diterpenes occur in coffee beans either as free alcohols or esterified to fatty acids at the C-17 position.



Intake levels of coffee diterpenes

Cafestol and kahweol are both present in Arabica beans. Robusta beans contain less cafestol and almost no kahweol, but they contain small amounts of 16-*O*-methylcafestol (Figure 1) [20]. Commercial roast and ground coffees contain about 1% (w/w) of diterpenes, of which 49% is cafestol, 47% is kahweol and 3% is 16-*O*-methylcafestol. Levels in decaffeinated coffee grounds are similar [21].

In unfiltered coffee brews, diterpenes are carried by oil droplets and floating bean particles. Diterpenes are well absorbed from oils [3,4,19]. The intake of 8 g of spent coffee grounds per day (providing 88 mg of diterpenes) for 3 weeks raised serum cholesterol levels by 0.65 mmol/l in volunteers [22], showing that coffee diterpenes are absorbed from floating particles as well. Diterpene measurements in coffee brews should thus include the contribution of the floating particles.

We applied a gas chromatography method for diterpene analysis [21] to various coffee brews. Boiled coffee as brewed in Scandinavia contained about 3 mg of cafestol, 4 mg of kahweol and negligible levels of 16-*O*-methylcafestol per cup of 150 ml [21]. We found similar diterpene levels in Turkish coffee and in coffee prepared in a plunger pot (also called a 'cafetiere'). In a controlled experiment, plunger pot coffee indeed raised serum LDL and VLDL levels in volunteers as much as boiled coffee [19]. Turkish coffee probably does the same. Espresso and mocha provided only 1–2 mg of each diterpene per cup [21], mainly because of the smaller serving sizes. Moderate mocha or espresso consumption will thus hardly affect serum lipid levels. Diterpene levels in soluble (instant), percolated and filtered coffee are about 0.1 mg of diterpenes per cup [21], and even heavy consumption will have negligible effects on serum lipid levels or coronary risk.

Excretion of coffee diterpenes in faeces

To estimate how much cafestol and kahweol is absorbed, we measured their excretion in faeces. Nine healthy male subjects ingesting 50–60 mg per day of each diterpene dissolved in oil for 4 weeks [19] collected faecal output for 96 h on dry ice (−80 °C) in the third week of supplementation. For each subject, faeces samples were homogenized with water (1:1), freeze-dried and analysed for diterpene content with HPLC. We found that on average 6% of the ingested amount of cafestol and 4% of kahweol was recovered from the faeces. Seven healthy subjects consuming fine coffee grounds providing 40–50 mg of each diterpene per day for 3 weeks [22] collected faeces for 120 h in the last week of supplementation. On average, 24% of cafestol and 26% of kahweol was recovered (Figure 2; R. Urgert and M. B. Katan, unpublished work). These figures suggest that diterpenes are well absorbed from oils and to a lesser extent also from grounds.

However, it is unknown whether some of the diterpenes may be lost during the procedure of freeze-drying. In addition, cafestol and kahweol may have been metabolized by the colonic flora into compounds that are no longer detectable with our standard chromatography method. Both phenomena will cause an overestimation of the absorbed amount of diterpenes. Studies with ileostomy patients, i.e. patients who have no colon, might produce more exact estimates of the absorbed amount of diterpenes.

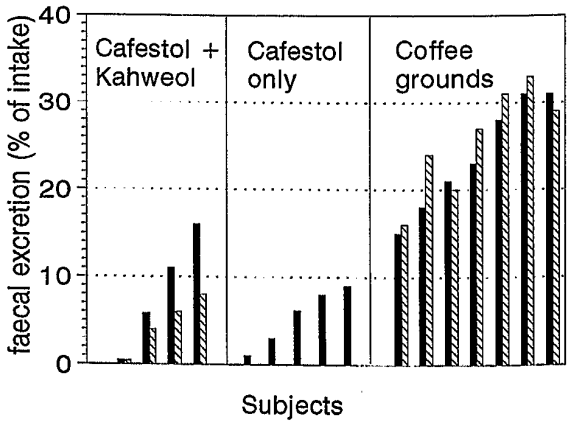
Excretion of coffee diterpenes in urine

Biotransformation of lipid-soluble xenobiotics occurs largely in the liver, and usually consists of two phases. The Phase I pathway converts the parent compound into more water-soluble

Figure 2

Daily excretion of cafestol (black bars) and kahweol (hatched bars) in faeces, expressed as the percentage of diterpenes consumed per day

Left and middle panel, diterpenes were purified from coffee oil, esterified to palmitic acid and dissolved in placebo oil. Four subjects swallowed capsules with 60 mg/day cafestol and 48 mg/day kahweol (left panel), and five others capsules with 63 mg of cafestol alone (middle panel). Faeces were collected on dry ice over 96 h in the third week of supplementation. Right panel, seven subjects consumed 8 g/day spent coffee grounds (providing 39 mg of cafestol and 49 mg of kahweol). Faeces were collected on dry ice for 120 h in the third week of supplementation.



metabolites. The Phase II pathway then conjugates the metabolite with a large hydrophilic moiety, such as glucuronic or sulphuric acid, to produce more water-soluble products that can be excreted by the kidneys.

We hypothesized that at least part of the diterpenes would not undergo Phase I metabolization, as they already possess accessible OH groups that permit conjugation (Figure 1). We found no free cafestol or kahweol in urine from subjects receiving diterpene-rich supplements [3,19,22], but treatment with β-glucuronidase which also had some sulphatase activity showed that up to 6% of ingested cafestol and 3% of ingested kahweol were excreted in the urine as simple conjugates of either glucuronic or sulphuric acid (Table 1; R. Urgert and M. B. Katan, unpublished work). The remainder of the ingested diterpenes is probably metabolized into compounds that are not detectable with our standard chromatography method, and may be excreted either by the gall bladder or by the kidneys.

Sites of action of coffee diterpenes

Effects on lipid metabolism

The mechanism by which coffee diterpenes influence lipid metabolism is largely unknown. The time course of the change in cholesterol is unusually long; 6 or more weeks are required to reach a new steady state [3]. Insight into metabolic control points of coffee diterpenes might thus lead to new ways to influence cholesterol levels in humans. In addition, although cafestol and kahweol seem to be unique for coffee beans [18], there may well exist related dietary compounds that have the same action.

Surprisingly, the effect of coffee diterpenes on lipid metabolism seems to be unique for *Homo sapiens*, as no effective animal model has yet been found. Boiled coffee elevated cholesterol levels in one study with hamsters [23], but attempts to verify this were unsuccessful [24,25]. Other experiments involving rats [24,26], gerbils [27] and rabbits [28] were also negative, regardless of the dosage, the mode of administration, the duration of treatment and the cholesterol content of the background diet. Cebus and Rhesus monkeys did not respond to the coffee oil that we had used in our human studies (Figure 3) [29]. It is possible that differences in absorption or metabolism of coffee diterpenes account for this curious species-specificity. However, the lack of an effect of coffee diterpenes in animals re-emphasizes the limitations of animal toxicological studies.

Cafestol – or any of its metabolites – might raise serum LDL and VLDL levels through an enhancement of lipoprotein synthesis in the liver. However, the serum concentration of the cholesterol precursor lathosterol, an indicator of cholesterol synthesis, was not markedly increased

Table 1

Urinary excretion of cafestol and kahweol as conjugates of glucuronic or sulphuric acid, in humans ingesting diterpene-rich supplements

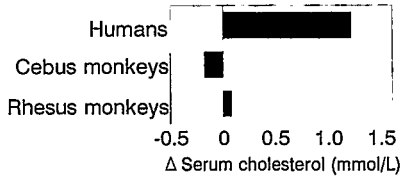
Excretion values are given as the mean (±S.D.) percentage of each diterpene consumed per day.

				Percentage of intake excreted as conjugate			
Supplement	*n*	Diterpene	Intake (mg/day)	After 2 weeks	After 3 weeks	After 4 weeks	After 6 weeks
Study 1							
Pure diterpenes [3]	2	Cafestol	73	6.4±1.9	–	4.4±0.4	2.5±0.1
	2	Kahweol	58	3.3±0.4	–	1.9±0.1	1.0±0.0
Study 2							
Pure diterpenes [19]	9	Cafestol	60–63	1.7±0.9	1.9±1.8	–	–
	4	Kahweol	48	0.3±0.1	0.7±0.5	–	–
Study 3							
Spent coffee grounds [22]	7	Cafestol	39	–	1.9±1.5	–	–
	7	Kahweol	49	–	0.5±0.6	–	–

Figure 3

Effect of coffee oil prepared by hexane extraction of spent coffee grounds on serum cholesterol levels in one human and two monkey studies

Humans ingested 0.03 g of coffee oil per kg body weight for 4 weeks in a placebo-controlled trial ($n = 12–16$ per group) [3]. Cebus monkeys ($n = 16$) ingested 0.18 g per kg body weight of either placebo or coffee oil in a cross-over study of 2×7.5 weeks, and Rhesus monkeys ($n = 6$) 0.20 g per kg body weight of either placebo or coffee oil in a cross-over study of 2×6 weeks [29].



after daily consumption of six cups of boiled coffee [9].

Decreased removal of LDL cholesterol from the bloodstream is an alternative explanation. LDL particles are taken up by LDL receptors, located on the cell surface, and mutations that reduce receptor synthesis cause familial hypercholesterolaemia. Cell studies of the effects of cafestol on LDL-receptor synthesis or activity produced contradictory results; cafestol decreased the uptake of LDL in human fibroblasts [30], but increased it in an intestinal cell line [31].

Effects on the liver

In our experiments with volunteers, each 2 mg of cafestol ingested per day raised the serum activity of alanine aminotransferase (ALT, formerly SGPT) by 1 unit/l [3]. Again, this effect was not present in monkeys that were fed the same coffee oil [29]. In humans, γ-glutamyltransferase (GGT) activity declined upon diterpene ingestion, but showed a rebound rise after withdrawal of the diterpenes [3,22].

The elevation of ALT activity points to mild perturbation of hepatocyte integrity by coffee diterpenes [32]. The activity of aspartate aminotransferase (AST, formerly SGOT) usually rises less on feeding coffee diterpenes than that of ALT [3,22]. Hepatic ALT is mainly cytoplasmic, whereas AST in liver cells is predominantly localized in mitochondria [32]. Possibly, coffee diterpenes or their metabolites disturb permeability of cell membranes in the liver parenchyma, but do not cause the extensive damage needed to release enzymes from mitochondria.

Could the hyperlipidaemic effects of coffee diterpenes be secondary to their effect on the liver? We found no consistent effects on serum bilirubin levels and alkaline phosphatase activities, which appears to exclude cholestasis. After withdrawal of the diterpene supplements in our experiments, the rises in serum ALT activity persisted longer than those in serum lipoproteins [3]. In addition, we found that both cafestol and kahweol actively elevated serum ALT activities, whereas cafestol had much more hyperlipidaemic activity than kahweol [19]. These findings make it improbable that the rises in serum lipid levels are coupled to the hepatocellular effect of coffee diterpenes.

Effects on the thyroid

Patients with hypothyroidism have raised serum levels of LDL and VLDL cholesterol, but not of high-density-lipoprotein cholesterol. These changes resemble those caused by diterpenes, but the levels of thyroxine, tri-iodothyronine and thyroid-stimulating hormone were all unaffected in 11 volunteers who took 2 g of coffee oil per day for 3 weeks, despite rises in LDL and VLDL cholesterol [33].

Effects on the kidney

We observed a consistent decline in serum creatinine levels of 5–10% in subjects consuming coffee oils [3], grounds [22] or purified diterpenes [19]. Possibly, the observed changes in GGT activity are related to effects on the kidney, as GGT also occurs in high concentrations in kidney tissue [32]. However, no difference in urinary output of creatinine was found between subjects consuming 8 g of coffee grounds per day and control subjects, and the mean serum creatine level was similar in 150 Norwegians who chronically consumed boiled coffee and in 159 matched filter-coffee drinkers (R. Urgert and M. B. Katan, unpublished work). These findings do not support an effect of coffee diterpenes on kidney function.

Effects on the colon

Supplements rich in coffee diterpenes reduced the frequency of adenocarcinoma of the colon in rats [34], which may be attributable to an induction of glutathione S-transferases by cafestol and kahweol [35]. Glutathione S-transferases are involved in the metabolism and detoxification of many xenobiotics. In humans, a lower incidence of colon cancer with coffee intake was also found

in some studies [36]. Possibly, coffee diterpenes that pass into the large bowel induce glutathione S-transferase in gastrointestinal cells, enhancing the detoxification of carcinogenic compounds in the colon. However, the relevance of coffee diterpenes in this respect is as yet hypothetical.

Conclusions

The coffee diterpenes cafestol and kahweol are well absorbed and may affect a number of organs in man. Their effects on blood lipid levels are well established and explain the cholesterol-raising effect of Scandinavian boiled coffee and other unfiltered coffee brews. A mild hepatotoxic effect is also plausible. All these effects are unique to *Homo sapiens* and do not occur in primates or rodents, but the cause of this curious species-specificity is not known.

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Chemoprotection against cancer by isothiocyanates and glucosinolates

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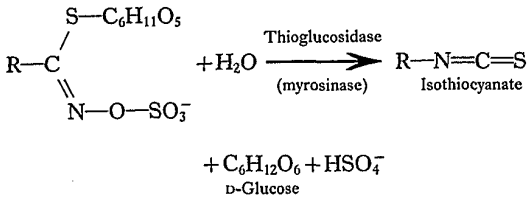
Introduction

Organic isothiocyanates (R—N=C=S) and glucosinolates, their biosynthetic precursors in plants, are attracting increasing attention as chemical and dietary protectors against cancer. More than 20 natural and synthetic isothiocyanates and several glucosinolates have been shown to block chemical carcinogenesis in animal models (see reviews in [1,2]); moreover, these substances are widely and often abundantly distributed in edible plants. The human consumption of isothiocyanates and glucosinolates is estimated at milligram quantities daily [3,4]. Nevertheless, it remains unclear to what extent these phytochemicals contribute to the well-recognized observations that individuals who consume large amounts of vegetables have lower risks of developing cancer (see review [5]). More than 100 isothiocyanates and glucosinolates have been isolated from plants, many of which belong to the family Cruciferae and more specifically to the genus *Brassica* (e.g. *Brassica oleracea* sp.: cabbage, cauliflower, Brussels sprouts, broccoli, kale) and the genus *Raphanus* (radishes and daikons) [3]. In addition, a large number of isothiocyanates have been synthesized for cancer chemoprotection studies [6,7].

Isothiocyanates are biosynthesized and stored in plants as relatively stable precursors known as glucosinolates (β-thioglucoside *N*-hydroxysulphates) [3]. The same plants also produce myrosinase (thioglucoside glucohydrolase, EC3.2.3.1) which normally is structurally segregated from its glucosinolate substrates, but is liberated when plant cells are damaged (by food preparation or eating) and promotes the hydrolysis of glucosinolates to isothiocyanates (as well as other products), hydrogen sulphate and glucose:



Chemistry and metabolism

Unlike glucosinolates, which are relatively stable and unreactive, isothiocyanates (R—N=C=S) contain a highly electrophilic central carbon atom that reacts rapidly under mild conditions with oxygen-, sulphur- or nitrogen-centred nucleophiles to give rise to thiocarbamates, dithiocarbamates or thiourea derivatives respectively [1]. Conjugates of isothiocyanates with GSH are especially important *in vivo* since they lead to the formation of dithiocarbamates which are the major products of isothiocyanate metabolism. Although such conjugations occur non-enzymatically, they are greatly accelerated by glutathione transferases (GSTs). These conjugations are catalysed by all cloned human GSTs tested, including GST A1–1, P1–1, M2–2 and M4–4 [8,9]. Although the conjugation with GSH is reversible and cleavage of the conjugates is also accelerated by GSTs, our studies indicate that the equilibria are quickly established and that the conjugation reaction is strongly favoured. The conjugates are rather poor sub-

Abbreviations used: DMBA, 7,12-dimethylbenz[*a*]-anthracene; glucobrassicin, indolylmethyl glucosinolate; glucosinalbin, 4-hydroxybenzyl glucosinolate; glucotropaeolin, benzyl glucosinolate; GST, glutathione transferase; NBMA, *N*-nitrosobenzylmethylamine; NMBA, *N*-nitrosomethylbenzylamine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; QR, quinone reductase.