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## Symposium on 'Functionality of nutrients and food safety'

# Validity of animal models for the cholesterol-raising effects of coffee diterpenes in human subjects

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Cafestol and kahweol, coffee lipids present in unfiltered coffee brews, potently increase LDL-cholesterol concentration in human subjects. We searched for an animal species in which cafestol similarly increases LDL-cholesterol. Such an animal model could be used subsequently as a model to study the mechanism of action of cafestol and kahweol. Cafestol and kahweol increased serum lipids in African green monkeys (*Cercopithecus aethiops*), cebus (*Cebus apella*) and rhesus (*Macaca mulatta*) monkeys, hamsters, rats and gerbils differently from the increase in human subjects. In African green monkeys, the rise in total cholesterol was less pronounced than that in human subjects. In addition, the increase in total cholesterol was predominantly due to a rise in HDL-cholesterol rather than LDL-cholesterol. Thus, the rise in plasma lipids might illustrate the mechanism in these monkeys rather than the mechanism in human subjects. In other animal species, cafestol and kahweol did not raise cholesterol consistently. The variability in effects on serum lipids could not be explained by the mode of administration or dose of diterpenes, nor by the amount of cholesterol in the diet. In conclusion, we did not find an animal model in which cafestol and kahweol elevate plasma lipoproteins to the same extent as in human subjects. For the time being, therefore, studies on the mechanism of action should be done preferably in human subjects.

**Animal models: Serum lipoproteins: Coffee: Cafestol and kahweol**

Unfiltered coffee brews markedly increase serum lipids in human subjects. The compounds responsible for this effect are cafestol and kahweol, which are present in coffee beans (Fig. 1; Heckers *et al*. 1994; Weusten-van der Wouw *et al*. 1994). The mechanism by which coffee diterpenes influence lipoprotein metabolism is largely unknown. An animal model with a response to cafestol and kahweol similar to that in human subjects would allow mechanistic studies that otherwise could not be done in human subjects. Here we review the use of animal models for understanding the action of cafestol and kahweol in human subjects.

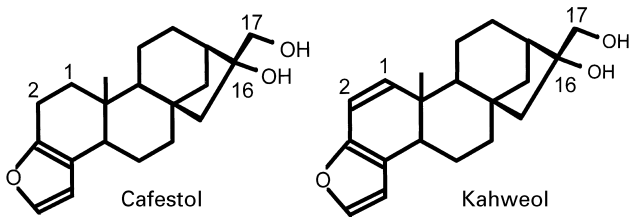


**Fig. 1.** Structure of the coffee diterpene alcohols cafestol and kahweol. In coffee brews, diterpenes are mainly esterified to fatty acids at the C-17 position.

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### Effect of coffee diterpenes on serum lipids in human subjects

The cholesterol-raising potential of unfiltered coffee depends mainly on its content of cafestol (Urgert *et al*. 1997). Unfiltered coffee brews such as Scandinavian boiled coffee, cafetiere (French press) coffee, and Turkish coffee contain about 3–6 mg cafestol per cup (Urgert *et al*. 1995; Gross *et al*. 1997). Filtered coffee does not contain cafestol, because coffee diterpenes are insoluble in water and do not pass through a paper filter (van Dusseldorp *et al*. 1991). In short-term studies each 10 mg cafestol ingested raised serum cholesterol an average of 0·13 mmol/l and serum triacylglycerols an average of 0·08 mmol/l after 4 weeks. Effects are linear up to 100 mg cafestol ingested/d (Urgert & Katan, 1997). In a long-term study the cafestol content of five cups of cafetiere coffee ingested per d caused a persistent rise in serum total cholesterol of 11–17 % and a rise in LDL-cholesterol of 9–14 % after 24 weeks (Urgert *et al*. 1996). Hence, about 80 % of the rise in total cholesterol was accounted for by LDL-cholesterol, and the remainder was due to a rise in VLDL-cholesterol. Cafetiere coffee did not affect HDL-cholesterol levels, although coffee diterpenes did slightly decrease HDL-cholesterol concentration in some previous studies (Zock *et al*. 1990; Weusten-van der Wouw *et al*. 1994; Urgert *et al*. 1997; Urgert & Katan, 1997). Serum triacylglycerols rose markedly (26 %) after 2–4 weeks of intake of cafetiere coffee. However, the rise in serum triacylglycerols subsided with chronic intake of coffee diterpenes (Urgert *et al*. 1996).

To study how cafestol affects serum lipoproteins in human subjects, we would like to use an animal model with a lipoprotein metabolism similar to that of human subjects. One criterion would be that the animal's response to dietary cholesterol is similar to that in human subjects. Rats, mice, dogs and squirrel monkeys (*Saimiri sciureus*) respond to dietary cholesterol by down-regulation of cholesterol synthesis and by up-regulation of bile acid production (i.e. cholesterol 7α-hydroxylase; *EC* 1.14.13.17) in the liver. As a consequence, plasma lipoprotein concentrations do not increase (Spady *et al*. 1985). In contrast, bile acid production is not affected when human subjects increase their cholesterol intake, while serum LDL- and HDL-cholesterol concentrations will increase. Hamsters, guinea-pigs, cynomolgus monkeys, cebus monkeys (*Cebus apella*), rhesus monkeys (*Macaca mulatta*), African green monkeys (*Cercopithecus aethiops*), baboons and pigs, like human subjects, have a low rate of cholesterol synthesis in the liver (Spady & Dietschy, 1983). In addition, these species do not increase bile acid synthesis when fed on cholesterol. Instead, liver cholesterol content increases, LDL-receptor activity is down-regulated and LDL-cholesterol concentration in the plasma increases (Spady *et al*. 1985). Thus, the response to dietary cholesterol in these animal species is similar to that in human subjects.

Male African green monkeys show many similarities to human subjects in the effects of dietary cholesterol and fatty acids on plasma lipoproteins and cholesterol metabolism (Rudel *et al*. 1986, 1990, 1991). Thus, we studied whether the African green monkey is also a good model for the effect of cafestol and kahweol on lipoprotein metabolism in human subjects. In addition, we will discuss whether we can use rhesus or cebus monkeys, hamsters, rats, gerbils or mice as a model to study the mechanism of action of the coffee diterpenes.

### Effect of coffee diterpenes in African green monkeys and human subjects

We fed eight male African green monkeys with a mean age of 15·9 years and a mean weight of 4·4 kg on a 'Western type' diet with an energy distribution of 17 % protein, 35 % fat (15 % saturated fat) and 48 % carbohydrate. Crystalline cholesterol (96 mg/MJ dietary energy) was added in order to produce total plasma cholesterol concentrations of 5–8 mmol/l, i.e. the range seen in human subjects. This diet was supplemented with either a placebo oil consisting of sunflower oil and palm oil (3:2, w/w), or with coffee oil. Both oils had a similar content of fatty acids (Terpstra *et al*. 1995). The diets provided 0·26 g coffee oil or placebo oil/kg body weight per d; as a result the coffee-oil diet provided 8 mg cafestol and 7 mg kahweol/kg body weight per d.

During a run-in period of 6 weeks, all monkeys consumed the placebo-oil diet. During the first treatment period of 7 weeks four animals were given the placebo-oil diet, while the other four received the coffee-oil diet. The two groups had similar initial cholesterol concentrations (6·41 (SD 2·76) *v*. 6·37 (SD 2·07) mmol/l). This period was followed by a wash-out period of 5 weeks, where only the placebo-oil diet was provided. These then followed a second treatment period of 7 weeks in which the placebo-oil diet and the coffee-oil diet were switched. Blood samples were taken in week 5 of the run-in period, in weeks 5, 6 and 7 of both treatment periods, and in week 5 of the wash-out period. In plasma we determined total cholesterol, LDL-cholesterol, HDL-cholesterol and triacylglycerols as described by Carr *et al*. (1993).

Food intake and body weight did not change during the experimental period. Coffee oil raised total plasma cholesterol by 14 %, LDL-cholesterol by 8 %, and HDL-cholesterol levels by 23 % (Table 1). About 32 % of the rise in cholesterol was accounted for by LDL-cholesterol and 57 % by HDL-cholesterol. We assume that the remaining 11 % was accounted for by VLDL- + IDL-cholesterol. Coffee oil increased plasma triacylglycerols by 35 % compared with placebo oil. Plasma levels of alanine aminotransferase (*EC* 2.6.1.2) did not differ between the placebo-oil and the coffee-oil periods (Table 1), which indicates that liver cell integrity was not disturbed. In two previous animal studies also, coffee diterpenes did not affect plasma alanine aminotransferase (Terpstra *et al*. 1995; Beynen *et al*. 1996).

The response of plasma cholesterol was higher in the four animals that consumed the coffee-oil diet in the second treatment period than in the four animals that consumed the coffee oil in the first treatment period: 26 % *v*. 2 % for total cholesterol; 21 % *v*. −5 % for LDL-cholesterol; 59 % *v*. −12 % for HDL-cholesterol respectively (Fig. 2). The reason for this finding could be the relatively short run-in period of 6 weeks on the high-fat high-cholesterol placebo-oil diet. In monkeys a steady-state in the proportions of fatty acids in

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**Table 1.** Plasma concentrations of total, LDL- and HDL-cholesterol, triacylglycerols and alanine aminotransferase (*EC* 2.6.1.2) in eight male African green monkeys after consumption of placebo oil or coffee oil for 7 weeks in a cross-over design*
(Mean values and standard deviations are shown for all eight monkeys, for the four COF-PL monkeys and for the four PL-COF monkeys)

| | | Placebo oil | | | | Coffee oil | | | | Coffee *v.* placebo oil | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Pretreatment | | Post-treatment | | Pretreatment | | Post-treatment | | | | |
| | Group | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Difference | 95 % CI | *P*† |
| Total cholesterol | All | 7·01 | 3·4 | 6·82 | 3·16 | 6·62 | 2·12 | 7·57 | 2·96 | 1·14 | 0·21, 0·92 | 0·03 |
| (mmol/l) | COF-PL | | | | | | | | | 0·40 | | |
| | PL-COF | | | | | | | | | 1·87 | | |
| LDL-cholesterol | All | 4·58 | 3·28 | 4·34 | 3·04 | 4·30 | 2·20 | 4·70 | 3·02 | 0·65 | –0·13, 0·77 | 0·09 |
| (mmol/l) | COF-PL | | | | | | | | | 0·17 | | |
| | PL-COF | | | | | | | | | 1·29 | | |
| HDL-cholesterol | All | 1·80 | 0·39 | 2·01 | 0·52 | 1·75 | 0·44 | 2·33 | 0·69 | 0·37 | –0·18, 0·54 | 0·14 |
| (mmol/l) | COF-PL | | | | | | | | | –0·23 | | |
| | PL-COF | | | | | | | | | 0·97 | | |
| Triacylglycerols | All | 0·28 | 0·16 | 0·27 | 0·09 | 0·30 | 0·14 | 0·42 | 0·27 | 0·13 | 0·00, 0·13 | 0·06 |
| (mmol/l) | COF-PL | | | | | | | | | 0·19 | | |
| | PL-COF | | | | | | | | | 0·07 | | |
| Alanine amino- | All | 46 | 15 | 47 | 17 | 47 | 29 | 50 | 31 | 2 | –5, 6 | 0·40 |
| transferase | COF-PL | | | | | | | | | 1 | | |
| (U/l) | PL-COF | | | | | | | | | 3 | | |

COF-PL, coffee-oil diet consumed first followed by placebo-oil diet; PL-COF, placebo-oil diet consumed first followed by coffee-oil diet.
* Pretreatment samples were obtained in week 5 of the run-in and wash-out period. Treatment samples were obtained in weeks 5, 6 and 7 of the placebo-oil or coffee-oil treatment period. For details of diets and procedures, see p. 552.
† Plasma lipids were different at the start of the treatment period with coffee or placebo oil due to carry-over effects. Thus, we calculated the response to coffee-oil or placebo-oil treatment by subtracting pretreatment values from the treatment values. The statistical signifance of differences in responses between the coffee or placebo oil treatment was tested using the one-tailed paired Student's *t* test.

the liver pools, in the distribution of non-esterified and esterified cholesterol in liver, in LDL-receptor activity in the liver, in LDL-cholesterol production, and in plasma LDL-cholesterol are reached after approximately 3 months of feeding on a high-cholesterol high-fat diet (Kris-Etherton & Dietschy, 1997). The four monkeys that consumed the coffee-oil diet in the second treatment period had consumed the placebo-oil diet for 18 weeks before they received the coffee-oil diet. Thus, the values for these latter four monkeys may give a better indication of the lipid-elevating effect of the coffee oil.

The monkeys received daily on average 35 mg cafestol and 31 mg kahweol at an energy intake of 2·21 MJ. The daily intake would have been 160 mg cafestol and 140 mg kahweol if the monkeys were fed on the same amount of energy as human subjects, i.e. 10 MJ/d. This amount of diterpenes is present in twenty-five cups of unfiltered coffee (Urgert & Katan, 1997), and it would raise serum cholesterol by an average of 2·05 mmol/l or 40 % and serum triacylglycerols by an average of 1·26 mmol/l or 120 % in human subjects (Urgert & Katan, 1997). The response of plasma cholesterol to coffee oil in African green monkeys was in the same direction, but markedly weaker than that in human subjects. Also, unlike human subjects, in the monkeys the rise in cholesterol was primarily due to increases in HDL-cholesterol rather than in LDL-cholesterol. Thus, the action of cafestol and kahweol on lipoprotein metabolism may illustrate that the mechanism in this monkey species is different from that in human subjects. These findings show that an animal species which is a good model to study the mechanisms of the effects of dietary cholesterol and fatty acids on lipoprotein metabolism may not necessarily be a good model to study the mechanism of the effect of cafestol and kahweol.

## Coffee diterpenes fail to show consistent results in other animal species

In contrast to the previously described findings in African green monkeys, coffee oil did not affect plasma cholesterol and triacylglycerols in cebus and rhesus monkeys (Terpstra *et al.* 1995). These monkeys consumed a daily amount of cafestol corresponding to that present in twelve to thirteen cups of boiled coffee per 10 MJ diet (Fig. 3). This dose is half that given to the African green monkeys, which might explain why the cebus and rhesus monkeys did not respond. In addition, the cebus and rhesus monkeys received a lower dose of dietary cholesterol (Fig. 3), which also might help to explain the lack of effect on serum lipids, because in various animal experiments the influence of fed components on serum cholesterol appeared greater against a dietary background rich in cholesterol (Beynen & West, 1989).

In male Syrian hamsters, coffee diterpenes had no consistent effect on serum lipids (Fig. 3). Boiled coffee elevated plasma cholesterol and triacylglycerols in one study with Syrian hamsters by Sanders & Sandaradura (1992), but our attempt to replicate this result was unsuccessful (Beynen *et al.* 1996). In another study by Ratnayake *et al.* (1995) coffee oil, a diterpene-rich fraction from coffee beans and purified diterpenes were also found to have no effect on serum lipids in hamsters, even though the animals received the same amount of dietary cholesterol and a much higher dose of cafestol than the hamsters in the study of Sanders & Sandaradura (1992). Paradoxically, serum cholesterol was

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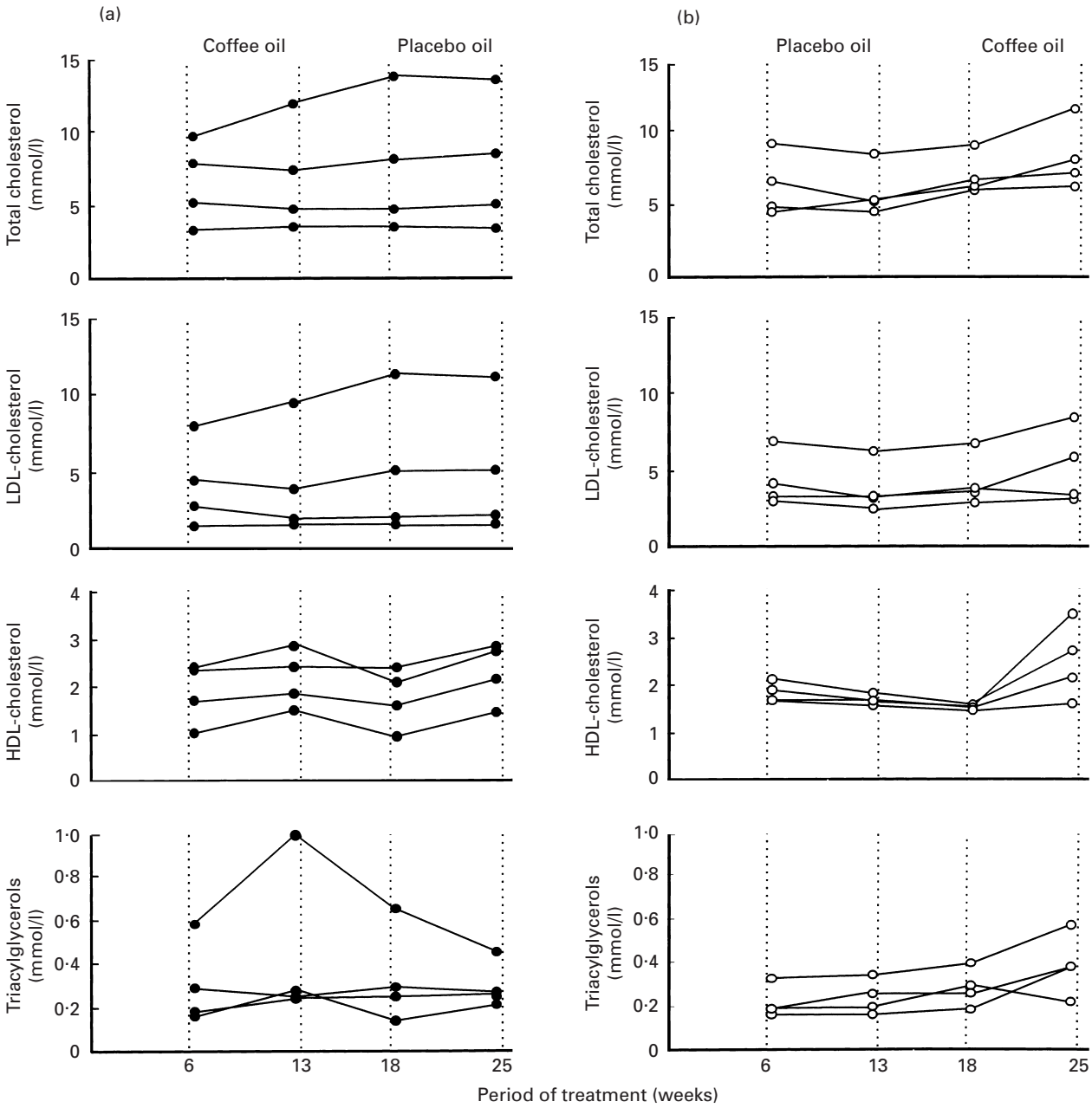


**Fig. 2.** Individual values for plasma total cholesterol, LDL- and HDL-cholesterol, and triacylglycerols in eight African green monkeys after a high-fat high-cholesterol diet supplemented with either placebo oil or coffee oil in a crossover design. Treatment with the placebo-oil diet or the coffee-oil diet lasted for 7 weeks. Treatments were separated by a wash-out period of 5 weeks. (●), Monkeys that first received the coffee-oil diet (a); (○), monkeys that first received the placebo-oil diet (b). For details of diets and procedures, see p. 552.

significantly increased when Ratnayake *et al.* (1995) repeated this experiment with a much lower dose of cafestol, dietary cholesterol and saturated fatty acids.

Rats also failed to show a consistent response of serum lipids to cafestol treatment (Fig. 3). Boiled coffee (Al-Kanhal *et al.* 1990) and coffee oil (AHM Terpstra, MB Katan, MPME Weusten-van der Wouw, B de Roos and AC Beynen, unpublished results) significantly increased serum cholesterol on both a low- and high-cholesterol diet. However, in two other studies using Wistar rats, serum lipids were not affected by unfiltered coffee (Hostmark *et al.* 1988; Beynen *et al.* 1996), although one of these studies used a background diet which was relatively high in cholesterol (Beynen *et al.* 1996).

Two studies in our laboratory have presented data on the effects of coffee in male gerbils (Fig. 3). Mensink *et al.* (1992) found that freeze-dried boiled coffee did not affect serum cholesterol in gerbils. However, the process of freeze-drying may have removed or modified the cafestol in the diets. In a second study we fed gerbils on coffee oil from the

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| Reference | Cafestol source | Effect: Mean | Effect: % increase from baseline | Cholesterol intake (mg/10 MJ per d) | Cafestol intake (cups of coffee/ 10 MJ per d) |
|---|---|---|---|---|---|
| (a) Human subjects | | | | | |
| Urgert *et al.* (1997) | Various | | 18 | 246 | 10 |
| (b) Monkey | | | | | |
| Terpstra *et al.* (1995) | Coffee oil | | −4 | 333 | 12 |
| | Coffee oil | | 2 | 590 | 13 |
| Present paper | Coffee oil | | 18 | 890 | 25 |
| (c) Hamster | | | | | |
| Sanders & Sandaradura (1992) | Coffee | | 17 | 514 | 2 |
| Mensink *et al.* (1992) | Coffee | | −17 | 293 | 7 |
| Ratnayake *et al.* (1995) | Oil | | −1 | 500 | 14 |
| | Coffee oil fraction | | −2 | 500 | 10 |
| | Diterpenes | | 13 | 500 | 11 |
| | Oil | | 15 | 188 | 4 |
| | Coffee oil fraction | | 3 | 188 | 3 |
| | Diterpenes | | 12 | 188 | 3 |
| Beynen *et al.* (1996) | Coffee | | 2 | 354 | 9 |
| | Coffee | | −9 | 59 | 9 |
| (d) Rat | | | | | |
| Hostmark *et al.* (1988) | Coffee | | −3 | <1 | 6 |
| Al-Kanhal *et al.* (1990) | Coffee | | 17 | 6367 | 7 |
| | Coffee | | 34 | <1 | 8 |
| Beynen *et al.* (1996) | Coffee | | −31 | 5000 | 2 |
| | Coffee | | 0 | 6 | 2 |
| Terpstra *et al.* (unpublished results)* | Oil | | 11 | 327 | 14 |
| | Oil | | 17 | 21 | 14 |
| (e) Gerbil | | | | | |
| Mensink *et al.* (1992) | Coffee | | 0 | 278 | 7 |
| Terpstra *et al.* (unpublished results)* | Oil | | 5 | 293 | 13 |
| | Oil | | 50 | 293 | 135 |
| | Oil | | −7 | 20 | 13 |
| | Oil | | 25 | 20 | 135 |

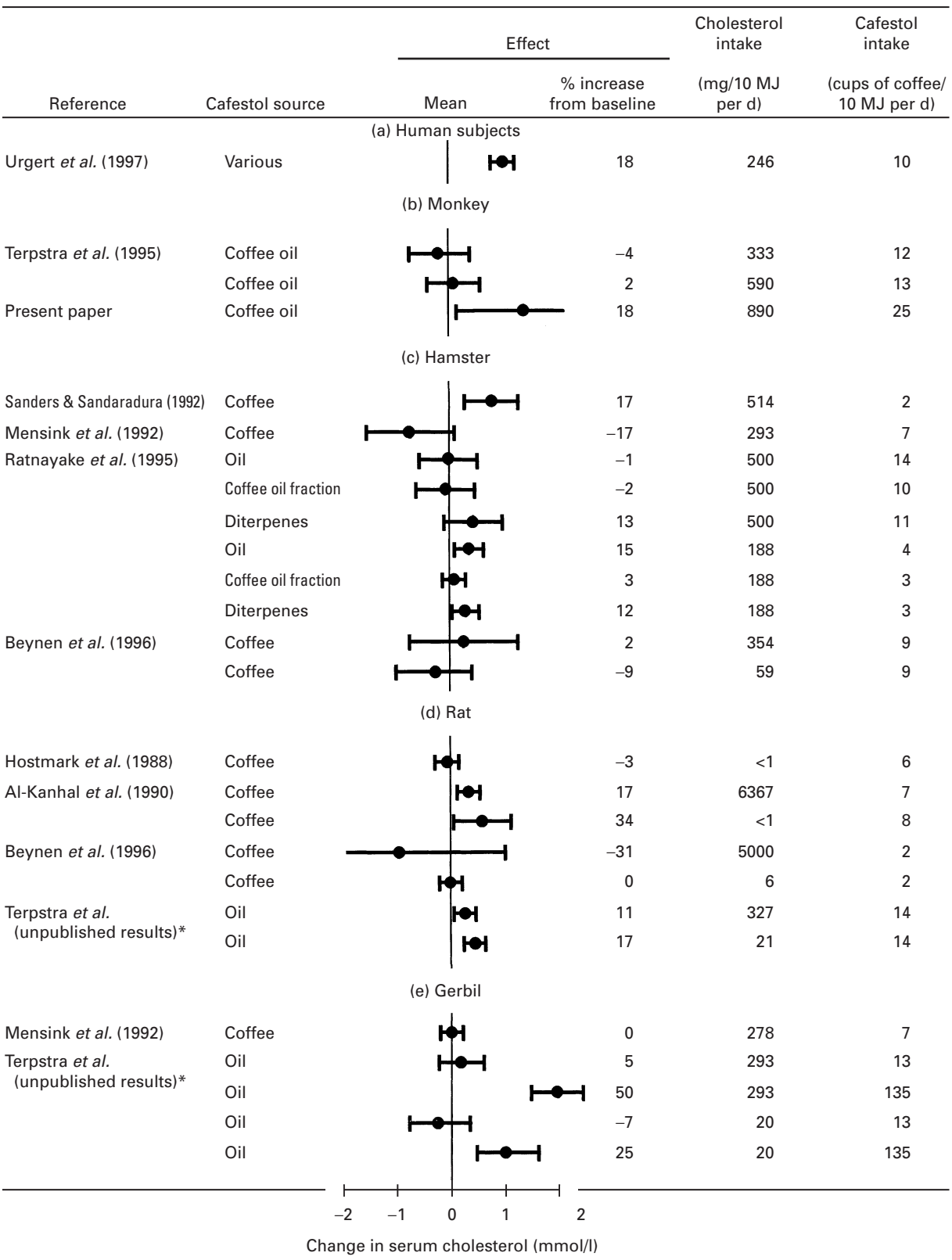


**Fig. 3.** Comparison of the effect of cafestol in human subjects with that in various animal species. Points represent means with 95 % CI represented by horizontal bars. Treatment periods varied from 2 to 20 weeks. Cafestol intake was recalculated to the amount of cups of coffee that would need to be consumed per 10 MJ per d, assuming that each 150 ml cup of unfiltered coffee contains 6·2 mg cafestol. The mean preparations of known diterpene content were given. *AHM Terpstra, MB Katan, MPME Weusten-van der Wouw, B de Roos and AC Beynen.

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same source and lot as we used in our human studies. Here, coffee oil significantly elevated plasma cholesterol compared with the placebo oil (AHM Terpstra, MB Katan, MPME Weusten-van der Wouw, B de Roos and AC Beynen, unpublished results).

We have also studied the effects of cafestol and kahweol on serum lipoproteins in hyperlipidaemic LDL-receptor knock-out mice and apolipoprotein E*3 Leiden mice (B de Roos, SM Post, M Vermeulen, L Afman, MC Jong, VEH Dahlmans, LM Havekes, F Stellaard, MB Katan and HMG Princen, unpublished results). A dose equivalent to eight or forty cups of unfiltered coffee/10 MJ per d raised serum cholesterol to the same extent in these mice species as in human subjects. The coffee diterpenes also raised serum cholesterol in the wild-type (C57BI/6) mice (B de Roos, SM Post, M Vermeulen, L Afman, MC Jong, VEH Dahlmans, LM Havekes, F Stellaard, MB Katan and HMG Princen, unpublished results). However, the rise in total cholesterol was due predominantly to a rise in VLDL- and IDL-cholesterol and not to a rise in LDL-cholesterol.

The form in which diterpenes were given (as pure compounds, as coffee oil or as unfiltered coffee) does not appear to explain the inconsistent effects on serum lipids in animals. Also the dose of coffee diterpenes does not explain the variability of the effects. Both high and low doses of diterpenes caused a significant rise in serum lipids in some animals (Fig. 4). In addition, a dietary background rich in cholesterol is neither necessary nor sufficient to produce a rise in serum cholesterol in animals (Fig. 4). As yet, there is no animal species in which cafestol and kahweol uniformly raised serum cholesterol. Thus, criteria such as study design and chance fluctuations might explain why cafestol raised serum cholesterol in some animal species, whereas other species did not respond to coffee diterpenes.

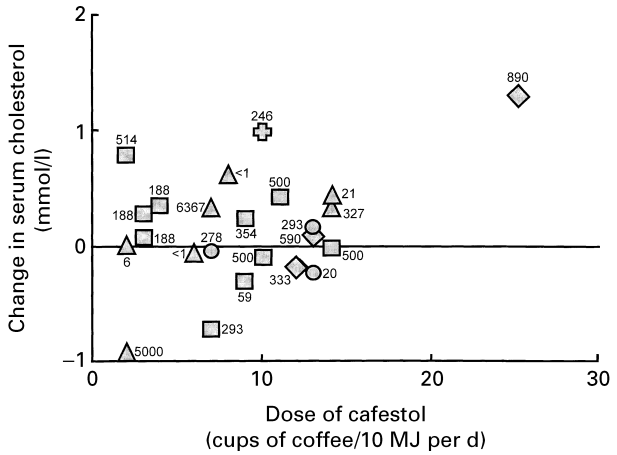


**Fig. 4.** Effect of cafestol intake (expressed as cups of coffee/10 MJ per d) on the response in serum cholesterol in various animal studies: (✚), human subjects; (◆), monkeys; (■), hamsters; (▲), rats; (●), gerbils. Responses were adjusted for the mean changes in the control group, if present. The values shown are the amounts of background dietary cholesterol (expressed in mg cholesterol/10 MJ per d) used in these studies.

## Conclusion

The effect of cafestol and kahweol on lipoprotein metabolism in monkeys, hamsters, rats and gerbils differs from that in human subjects. In African green monkeys, coffee diterpenes appeared to raise HDL-cholesterol as much as LDL-cholesterol, although the rise was not statistically significant for either lipoprotein. The small group size and time on the diet appeared to limit the significance of the outcome. In the other animal species, cafestol and kahweol did not raise serum cholesterol consistently and to the same extent as in human subjects. We will gain little knowledge by extrapolating these effects of cafestol and kahweol on plasma lipoproteins in animals to human subjects unless the animal model has first been shown to respond to the intervention in the same way as human subjects. For the time being, studies on the mechanism of action should be done preferably in human subjects.

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