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Coffee bean extracts rich and poor in kahweol both give rise to elevation of liver enzymes in healthy volunteers

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Abstract

Background: Coffee oil potently raises serum cholesterol levels in humans. The diterpenes cafestol and kahweol are responsible for this elevation. Coffee oil also causes elevation of liver enzyme levels in serum. It has been suggested that cafestol is mainly responsible for the effect on serum cholesterol levels and that kahweol is mainly responsible for the effect on liver enzyme levels. The objective of this study was to investigate whether coffee oil that only contains a minute amount of kahweol indeed does not cause elevation of liver enzyme levels.

Methods: The response of serum alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) to Robusta coffee oil (62 mg/day cafestol, 1.6 mg/day kahweol) was measured in 18 healthy volunteers.

Results: After nine days one subject was taken off Robusta oil treatment due to an ALAT level of 3.6 times the upper limit of normal (ULN). Another two subjects stopped treatment due to other reasons. After 16 days another two subjects were taken off Robusta oil treatment. One of those subjects had levels of 5.8 ULN for ALAT and 2.0 ULN for ASAT; the other subject had an ALAT level of 12.4 ULN and an ASAT level of 4.7 ULN. It was then decided to terminate the study. The median response of subjects to Robusta oil after 16 days was 0.27 ULN (n = 15, 25^{th},75^{th} percentile: 0.09;0.53) for ALAT and 0.06 ULN (25^{th},75^{th} percentile -0.06;0.22) for ASAT.

Conclusions: We conclude that the effect on liver enzyme levels of coffee oil containing hardly any kahweol is similar to that of coffee oil containing high amounts of kahweol. Therefore it is unlikely that kahweol is the component of coffee oil that is responsible for the effect. Furthermore, we conclude that otherwise unexplained elevation of liver enzyme levels observed in patients might be caused by a switch from consumption of filtered coffee to unfiltered coffee.

Background

Consumption of unfiltered coffee types raises serum cholesterol levels in humans [1-4]. Unfiltered coffee also causes elevated liver enzyme levels in serum. Cafestol and kahweol are responsible for the effect of unfiltered coffee on serum cholesterol. These diterpenes are present in the oil derived from coffee beans. The only difference between cafestol and kahweol is a double bond present between the C1–C2 atoms in kahweol.

Two types of coffee beans are used for brewing coffee: Arabica and Robusta. Arabica beans contain both cafestol and kahweol, whereas Robusta beans contain half as much cafestol and hardly any kahweol [5]. Cafestol raises serum cholesterol more potently than kahweol does. A mixture of cafestol (60 mg/day) and kahweol (51 mg/day) increased serum cholesterol only slightly more than pure cafestol (64 mg/day) did [3]. Results with pure kahweol are not available due to difficulties with purification and stability of this diterpene.

Coffee oil also raises serum levels of the liver enzyme alanine aminotransferase (ALAT) and to a lesser extent aspartate aminotransferase (ASAT). Elevation of these liver enzymes may indicate injury of hepatocytes [6-8]. For example in acute hepatitis, either viral or drug-induced, both ALAT and ASAT are elevated. The ALAT levels often exceed the ASAT levels. ALAT is predominantly present in the cytosol of hepatocytes and ASAT is predominantly present in the mitochondria. When hepatocytes sustain damage to their membranes ALAT is released from the cytosol, whereas when hepatocytes sustain more severe damage ASAT is released from the mitochondria [6-8]. When ASAT levels are more increased than ALAT levels; this could indicate obstruction of the bile duct or alcohol abuse.

We have earlier suggested that kahweol is mainly responsible for the effect of coffee oil on liver enzyme levels [3,9]. A mixture of cafestol and kahweol raised liver enzyme levels more potently than pure cafestol, whereas the effect on serum cholesterol levels is similar. This would suggest that the structural difference between cafestol and kahweol causes these diterpenes to act on different pathways in the liver. On the basis of the suggestion that kahweol is mainly responsible for the effect on liver enzyme levels, we hypothesized that Robusta oil, which contains a negligible amount of kahweol, would induce no or a smaller response of liver enzymes than Arbica oil, while maintaining its cholesterol-raising effect. In order to test this hypothesis we designed a study in which healthy volunteers consumed Robusta oil.

Methods

Subjects

Subjects were recruited among the student population of Wageningen, a university town in the Netherlands. Twenty-one volunteers were included; their health was assessed by means of a questionnaire, and blood and urine testing. We used the following eligibility criteria: serum cholesterol < 8 mmol/l, serum triglycerides < 3.0 mmol/l, no glucosuria, normal liver enzyme activities in serum, normal bilirubin levels in serum, no use of medication with effects on serum lipids, no consumption of unfiltered coffee, and no history of gastrointestinal or liver disease. The following enzyme activities were measured in serum: ALAT, ASAT, alkaline phosphatase, amylase, γ-glutamyltranspeptidase and lactate dehydrogenase. Three subjects were excluded at baseline due to serum bilirubin concentrations above the upper limit of normal. Thus, eighteen subjects were enrolled in the study. The Medical Ethics Committee of Wageningen University and Research Centre approved the study. It was determined a priori that subjects would be taken off treatment as soon as ALAT levels exceeded 2.5 times upper limit of normal or ASAT levels exceeded 1.5 times the upper limit of normal. Each volunteer gave an informed consent.

Study design

Green robusta coffee beans were roasted and ground. Robusta oil was extracted by hexane extraction under food-grade conditions at the Agrotechnology & Food Innovations institute in Wageningen. To make ingestion more convenient the oil was administered as an emulsion containing 50% Robusta oil with 50% water. Subjects consumed 2 ml of Robusta oil twice a day for four weeks resulting in a daily dose of 62 mg cafestol and 1.6 mg kahweol (samples analyzed using DIN 10779, 1999).

After two days of Robusta oil consumption the first blood sample was taken for determination of liver enzyme activities. From then on blood was drawn every seven days and liver enzyme activities were determined in the serum within 24 hours. ALAT (Alanine Aminotransferase Flex reagent cartridge, Dade Behring) and ASAT (Aspartate Aminotransferase Flex reagent cartridge, Dade Behring) were measured at 37°C. Liver enzyme activities are given in multiples of the upper limit of normal because activities vary with the temperature used when determining liver enzyme activities in different laboratories. The upper limits of normal at this laboratory were 45 IU/l for ALAT and 50 IU/l for ASAT.

Subjects were asked to maintain their lifestyles and dietary habits for the duration of the study. They reported the amount of coffee oil taken daily and changes in diet, smoking, physical activity, use of medication, and illness, in diaries.

Statistical analysis

To calculate the effect of Robusta oil on liver enzyme levels we subtracted baseline levels from levels after treatment for each subject. We tested the differences between baseline and treatment levels with the Wilcoxon signed-rank test. We present median differences with the 25^{th} and 75^{th} percentile. The responses of serum cholesterol and triglycerides were also calculated as the level after treatment minus the baseline level for each subject. Serum lipid responses are presented as means with 95% confidence intervals (CI95%)

We used Bayesian statistics to combine existing evidence of the effect of coffee oil on levels of serum cholesterol with the present data. The Bayes factor was derived from the *P* value of the Student's *t* test: Bayes factor = $exp(-Z^2/2)$, where *Z* is the *Z* score that corresponds to the *P* value obtained with the Student's *t* test. A priori probabilities were converted to a priori odds and multiplied by the Minimum Bayes factor to obtain a posteriori odds. Finally the a posteriori odds were converted to a posteriori probabilities [10].

P values reflect the probability of obtaining the observed results or more extreme ones under the null hypothesis. A priori probabilities reflect our a priori estimation of the probability of the null hypothesis on the basis of the current literature. A posteriori probabilities reflect the probability of the null hypothesis when combining a priori evidence with evidence from the current study. We assigned an a priori probability of 10% to the null hypothesis that the effect of Robusta oil on serum cholesterol levels was equal to zero. The rationale for this postulation is that the effect of coffee oil on serum cholesterol is well established [1-4].

Results

Eighteen subjects were enrolled in the study, three men and 15 women. Figure 1 shows the number of subjects that were screened, enrolled, and excluded. Table 1 provides baseline characteristics of the enrolled subjects. During the study two subjects withdrew: one because of an infectious disease and the other because of the taste of the coffee oil. After nine days one subject was taken off treatment due to an ALAT level of 3.6 times the upper limit of normal (ULN). After 16 days another two subjects had to stop due to elevated ALAT and ASAT levels. One of those subjects had a level of 5.8 ULN for ALAT and 2.0 ULN for ASAT; the other subject had an ALAT level of 12.4 ULN and an ASAT level of 4.7 ULN. It was then decided to terminate the study, as prescribed by the study protocol: Three subjects or more showing a liver enzyme level above 2.5 times upper limit of normal for ALAT or 1.5 times the upper limit of normal for ASAT.

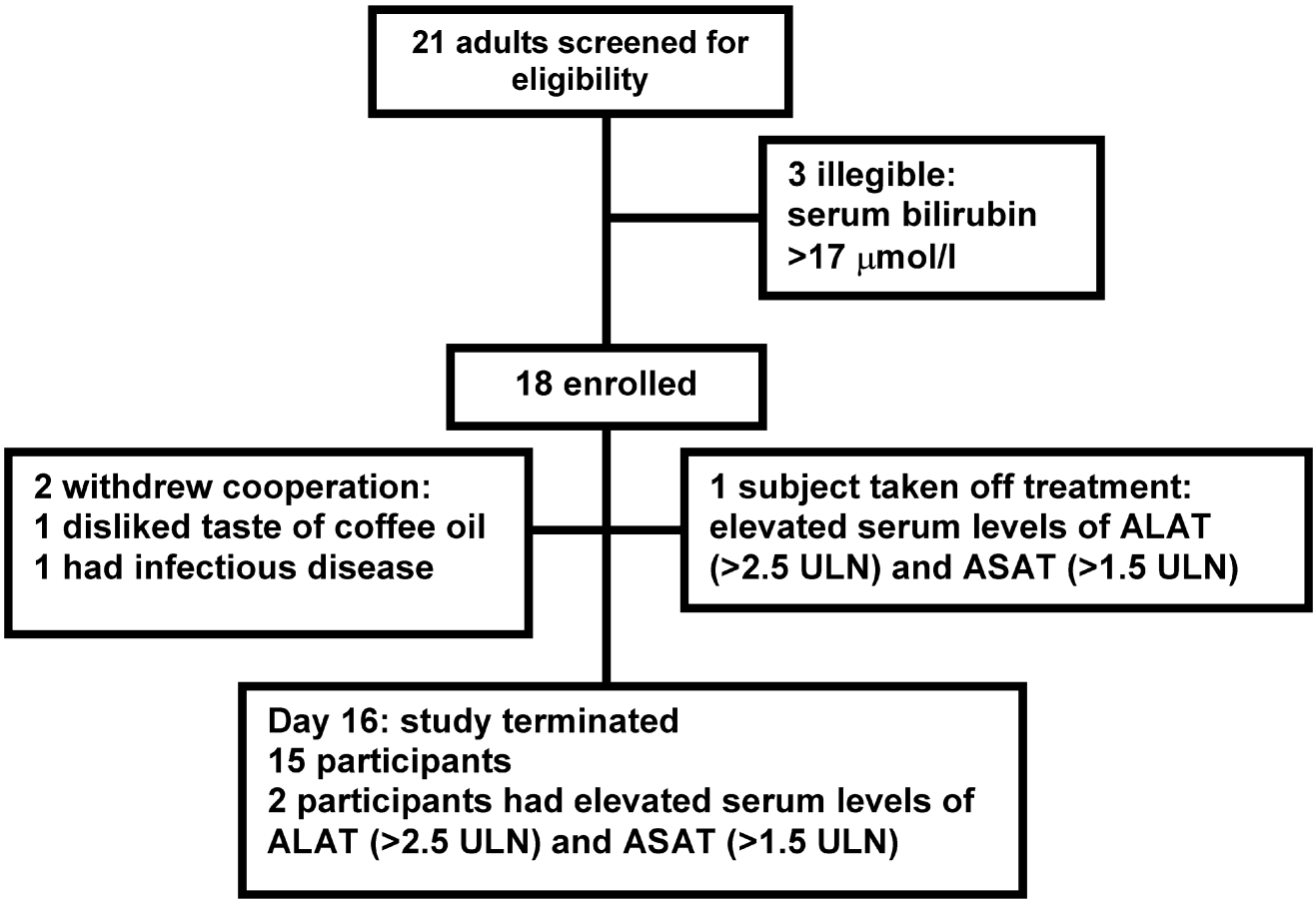

Figure 1
Diagram of the number of subjects that were screened, enrolled, and excluded. ULN = times the upper limit of normal.

Table 1: Baseline characteristics for all subjects who started the study

Characteristic	(n = 18)
Age (years)	22 ± 5
Height (m)	1.73 ± 0.09
Weight (kg)	64.3 ± 10.3
Body mass index (kg/m^2)	21.3 ± 1.6
Serum total cholesterol (mmol/l)	4.4 ± 1.0
Serum triglycerides (mmol/l)	0.85 ± 0.38
Alanine aminotransferase (IU/l)	20 ± 8
Aspartate aminotransferase (IU/l)	16 ± 4
Current smokers n (%)	2 (11)
Alcohol (glass/week) median (25th percentile, 75th percentile)	8 (2, 15)

Variables presented as mean ± sd, current smokers presented as n (%), and alcohol consumption as median with the 25th and 75th percentile.

Response of liver function parameters to Robusta oil

The median response after 16 days of coffee oil consumption was an increase of 0.27 ULN (25th,75th percentile:0.09,0.53) for ALAT and 0.06 ULN (25th,75th percentile: -0.06,0.22) for ASAT.

Table 2 shows levels of liver function parameters for all subjects that received Robusta oil treatment for 16 days. Follow-up measurements took place at termination, and after four and eight weeks. After four weeks two subjects had ALAT levels above normal. One of those subjects showed a large response of ALAT after 16 days of treatment, the other subject did not show a response above the upper limit of normal during the treatment period. After eight weeks ALAT and ASAT levels of all subjects were within normal limits again. We observed no significant effect of Robusta oil on alkaline phosphatase, amylase, bilirubin, γ-glutamyltranspeptidase and lactate dehydrogenase levels after 16 days of Robusta oil consumption. However, during the follow-up measurement four weeks after termination we observed increased levels of serum bilirubin and decreased levels of alkaline phophatase. Compared to baseline values bilirubin was increased by 3.0 μmol/l (25th,75th percentile: -2.5,6) and alkaline phosphatase was decreased by 0.05 ULN (25th,75th percentile:-0.16,-0.01).

Serum lipid response to Robusta oil

Total serum cholesterol levels were raised 0.27 mmol/l (n = 15, CI95% -0.11;0.64) after 16 days of coffee oil treatment. According to the Student's *t* test this effect was not significantly different from 0 at the $p < 0.05$ level. However, Bayesian analysis showed that the evidence from this study actually reduces an a priori probability for no effect on cholesterol of 10% to an a posteriori probability of 4%. See table 3 for the Bayesian analysis including the posteriori probabilities at several a priori probabilities. Serum triglycerides were elevated by 0.46 mmol/l (CI95% 0.26;0.66) in the 15 subjects who were in the study after 16 days.

Discussion

Robusta oil caused a rise of ALAT levels of more than 2.5 times the upper limit of normal in three out of eighteen subjects (17%). In our previous study with Arabica oil we observed ALAT levels of more than 2.5 times the upper limit of normal in eight out of 50 subjects (16%) in the first period and in five of 40 subjects (13%) not in the first, but only in a second treatment period [11]. Therefore we conclude that the effect of Robusta oil on liver enzyme levels is similar to that of Arabica oil. Levels of ASAT were less affected by coffee oil than ALAT levels. ALAT is predominantly present in the cytosol of hepatocytes, whereas ASAT is predominantly present in the mitochondria. This could mean that the outer membranes of hepatocytes have become leaky but that the cells are still largely intact. When hepatocytes sustain more severe damage, the serum levels of ASAT would exceed those of ALAT [6-8]. ALAT levels were also elevated in subjects after daily consumption of unfiltered coffee for six months [12], but were not elevated in life-long consumers [1,13]. This suggests that the effect of unfiltered coffee on ALAT levels is transient when consumed over long periods of time and that possibly an adaptation mechanism is present.

Another marker of liver damage, γ-glutamyltranspeptidase (γGT), has been shown to decrease during consumption of boiled coffee. After withdrawal of treatment with coffee lipids or coffee oil an rebound increase above baseline values in serum levels of γGT is observed [1,3,14,15]. In the present study we observed a 14% decrease in serum activities of γGT during treatment and an increase of 17% compared to baseline eight weeks after termination. These effects are not statistically significant in the present study. This is due to the limited number of subjects and the short duration of the coffee oil treatment. We also observed a

Table 2: levels of liver parameters at baseline after Robusta oil treatment and during follow-up

Parameter	Normal limits	Baseline	Treatment	Follow-up1	Follow-up 2
		n = 15	n = 15	n = 14	n = 14
Alanine aminotransferase	< 45 IU/l	0.38 [0.36,0.56]	0.67 [0.58,1.27]**	0.49 [0.39,0.72]*	0.39 [0.35,0.53]
Aspartate aminotransferase	< 50 IU/l	0.32 [0.26,0.38]	0.44 [0.26,0.56]	0.32 [0.28,0.42]	0.38 [0.28,0.41]
Alkaline phosphatase	40 – 125 U/l	0.45 [0.36,0.58]	0.43 [0.35,0.54]	0.42 [0.34,0.51]*	0.49 [0.37,0.53]
Amylase	35 – 130 U/l	0.50 [0.45,0.61]	0.48 [0.38,0.52]	0.49 [0.46,0.58]	0.47 [0.42,0.57]
Bilirubin	< 17 umol/l	0.47 [0.41,0.65]	0.53 [0.35,0.65]	0.65 [0.57,0.82]**	0.53 [0.40,0.74]
γ-glutamyltranspeptidase	< 40 U/l for women < 75 U/l for men	0.29 [0.24,0.38]	0.25 [0.20,0.45]	0.31 [0.27,0.51]	0.34 [0.25,0.46]
Lactate dehydrogenase	230 – 485 IU/l	0.60 [0.56,0.61]	0.61 [0.49,0.65]	0.60 [0.50,0.64]	0.54 [0.52,0.65]

All values are medians in units of times the upper limit of normal with the 25th and 75th percentile between brackets. Treatment values were obtained after 16 days of Robusta oil treatment. Follow-up 1 took place four weeks after termination of the intervention and Follow-up2 eight weeks after termination. * value differs significantly from baseline ($p < 0.05$) in the Wilcoxon signed-rank test. ** $p < 0.01$

Table 3: Change in prior probabilities of cafestol not affecting serum cholesterol to posterior probabilities using data of the present study and Bayesian analysis

Prior probability	Prior odds (Yes/No)	Posterior odds	Posterior probability
0.90 (very strong)	0.9/(1-0.9) = 9	9x Bayes factor = 3.22	3.22/(1+3.22) = 0.76
0.75 (strong)	0.75/(1-0.75) = 3	3x Bayes factor = 1.07	1.07/(1+1.07) = 0.52
0.50 (equivocal)	0.50/(1-0.50)= 1	1x Bayes factor = 0.36	0.36/(1+1.07) = 0.26
0.25 (weak)	0.25/(1-0.25) = 0.33	0.33x Bayes factor = 0.12	0.12/(1+0.12) = 0.11
0.10 (very weak)	0.10/(1-0.10) = 0.11	0.11x Bayes factor = 0.04	0.04(1+0.04) = 0.04

A priori probabilities were converted to a priori odds and multiplied by the minimum Bayes factor*. The obtained a postiori odds were converted to a postiori probabilities. *Bayes factor = e to the power $-Z^2/2$, where Z is the Z-score corresponding to the P-value for obtaining an effect of 0.27 mmol/l under the null hypothesis. P-value = 0.15, Z-score = 1.43 the minimum Bayes factor = 0.36

7% decrease in serum activities of alkaline phosphatase compared to baseline four weeks after termination. A tendency of alkaline phosphatase to be decreased during coffee lipid treatment was observed in previous studies [1,3]. Bilirubin levels were 38% increased during the follow-up measurement after four weeks compared to baseline. We observed no effect of Robusta oil treatment on amylase, a marker for pancreatitis, or lactate dehydrogenase, which is used for diagnosis of heart, muscle, and liver diseases. Increases of γGT and bilirubin after stopping treatment with coffee oil could indicate cholestasis. Cholestasis is functionally defined as a disruption in secretion of bile acids from the liver. Disrupted secretion causes elevated levels of bile acids in the liver, which causes damage to the hepatocytes. However, in cholestatic disease alkaline phosphatase is strongly increased, which is not the case with coffee oil treatment.

In Scandinavia, where large amounts of unfiltered coffee were commonly consumed, risk of coronary heart disease is high and was associated with consumption of unfiltered coffee but mortality rates of liver cirrhosis have been typically low [16]. Therefore, it is unlikely that consumption of unfiltered coffee produces severe damage to the liver. However, it is possible that unfiltered coffee can cause sub-clinical hepatic injury in some individuals. At present we have no evidence that the changes in liver enzyme levels induced by coffee oil or unfiltered coffee are of clinical relevance. Because we have no liver biopsies from subjects we are not able to demonstrate possible liver damage *in vivo*. No results from animal studies showing the effect of coffee oil on the liver have been published.

Interestingly, cafestol and kahweol have been shown to upregulate detoxification pathways in the liver of rat and mice and human cultured cells [17-21]. This effect on detoxification is hypothesized to explain the observed inverse association between coffee consumption and certain cancer types [20,22-24]. Possibly, cafestol upregulates these pathways due to its toxicity and as a "side effect" enhances detoxification of carcinogenic compounds.

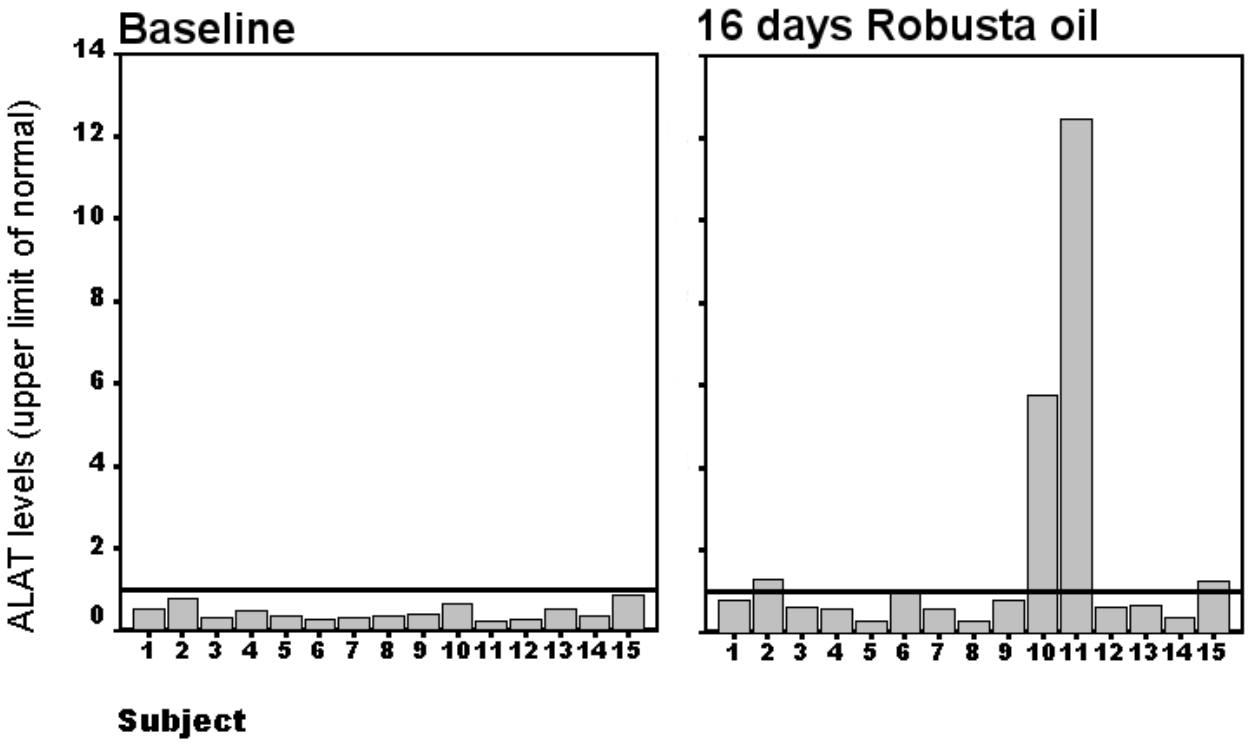

Figure 2
ALAT levels of subjects at baseline and after 16 days of Robusta oil consumption. The horizontal line indicates the upper limit of normal.

After 16 days of Robusta oil treatment serum cholesterol levels were elevated but this effect was not statistically significant at the $P < 0.05$ level according to the conventional frequentist analysis. This was expected from previous studies: the full effect on serum cholesterol is only observed after 4–6 weeks of coffee oil consumption. After four weeks daily consumption of 62 mg of cafestol results in a rise in serum cholesterol of 0.8 mmol/l. The Bayesian analysis, however, indicates that the present study in fact reinforces the existing evidence that coffee oil raises serum cholesterol, independent of how strong one judges this prior evidence to be (Table 3). We also found that triglycerides were elevated after 16 days of treatment, which was also expected from previous studies [1-4].

On the basis of our results it is not likely that it is kahweol, which is mainly responsible for the effect of coffee oil on liver enzyme levels, as has been suggested in two previous studies [3,9]. In most subjects coffee oil caused elevation of liver enzyme levels. However, this elevation was more extreme in a small number of subjects. Figure 2 shows ALAT levels and figure 3 shows ASAT levels of subjects at baseline and after 16 days of coffee oil consumption. Although no parallel placebo group was present in this study it is unlikely that such large responses of liver enzymes would be observed with placebo oil. This is supported by previous studies with coffee and placebo oil [1,9,25]. We found no correlation between the response of liver enzymes to coffee oil and baseline liver enzyme activities, serum lipid response, or alcohol. Moreover, a previous study showed that the liver enzyme response is not consistent within subjects [11]. In this study we also found no correlation between alcohol intake and liver enzyme response. Although we cannot rule out the possibility that alcohol affected the liver enzyme response during the study it does not seem likely that alcohol intake could fully explain the observed increases in ALAT and ASAT. Furthermore, γGT is decreased rather than increased during consumption of coffee oil, whereas alcohol causes increases in γGT levels.

Together with the observation that most subjects do not show such a large increase in liver enzyme levels during coffee oil treatment, this suggests that an unknown environmental factor enhances the response of liver enzymes to coffee oil in a number of subjects. We conclude that increased ALAT or ASAT activities in patients may be caused by a switch from filtered to unfiltered coffee. If

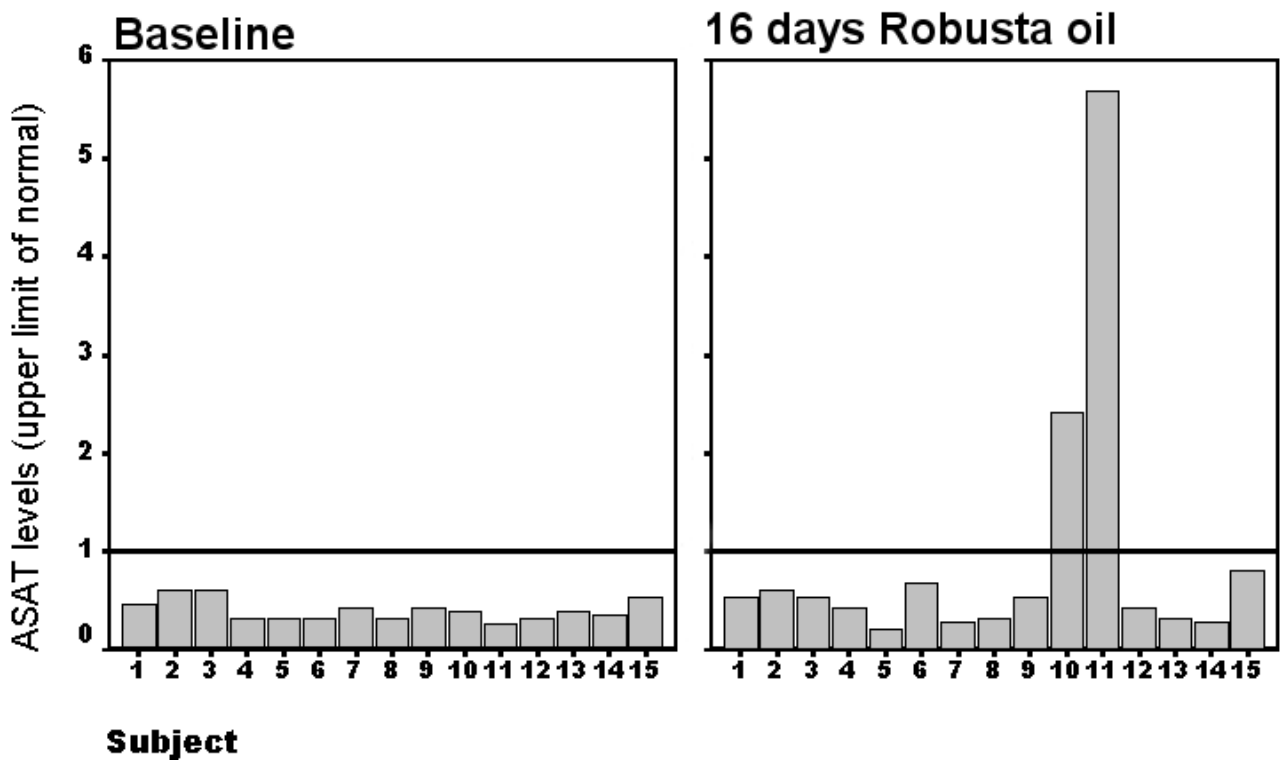

Figure 3
ASAT levels of subjects at baseline and after 16 days of Robusta oil consumption. The horizontal line indicates the upper limit of normal.

raised activities of ALAT and ASAT are caused by a change in coffee consumption, the ratio ASAT/ALAT will be smaller then 1 and other markers of liver damage such as γGT and alkaline phophatase will be typically within normal limits. When otherwise unexplained elevation of ALAT and ASAT activities are observed it would be advisable to ask a patient if he/she consumes large amounts of unfiltered coffee such as French press coffee.

Author's contributions

MVB participated in designing and planning the study, headed the investigation during the intervention period, analyzed the data and wrote the paper. EGS participated in designing the study, contributed to analysis of the data and interpretation of the results. MBK was the senior scientist supervising the project.

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References

1. Weusten-Van der Wouw MP, Katan MB, Viani R, Huggett AC, Liardon R, Lund-Larsen PG, Thelle DS, Ahola I, Aro A, *et al.*: **Identity of the cholesterol-raising factor from boiled coffee and its effects on liver function enzymes.** *J Lipid Res* 1994, **35:**721-733.
2. Urgert R, Katan MB: **The cholesterol-raising factor from coffee beans.** *Annu Rev Nutr* 1997, **17:**305-324.
3. Urgert R, Essed N, van der Weg G, Kosmeijer-Schuil TG, Katan MB: **Separate effects of the coffee diterpenes cafestol and kahweol on serum lipids and liver aminotransferases.** *Am J Clin Nutr* 1997, **65:**519-524.
4. Urgert R, Schulz AGM, Katan MB: **Effects of cafestol and kahweol from coffee grounds on serum lipids and serum liver enzymes in humans.** *Am J Clin Nutr* 1995, **61:**149-154.
5. Viani R: **Coffee.** In *Ullmann's Encyclopedia of Industrial Chemistry* Weinheim, Germany:VCH Verlag; 1986:315-339.
6. Keil E: **Determination of enzyme activities in serum for the detection of xenobiotic effects on the liver.** *Exp Pathol* 1990, **39:**157-164.
7. Herrera JL: **Abnormal liver enzyme levels. The spectrum of causes.** *Postgrad Med* 1993, **93:**113-116.
8. Sherman KE: **Alanine aminotransferases in clinical practice. A review.** *Arch Intern Med* 1991, **151:**260-265.
9. van Rooij J, van der Stegen GHD, Shoemaker RC, Kroon C, Burggraaf J, Hollaar L, Vroon TFFP, Smelt AHM, Cohen AF: **A placebo-controlled parallel study of the effect of two types of coffee oil on serum lipids and transaminases: identification of chemical substances involved in the cholesterol-raising effect of coffee.** *Am J Clin Nutr* 1995, **61:**1277-1283.
10. Goodman SN: **Toward evidence-based medical statistics 2: The Bayes factor.** *Ann Intern Med* 1999, **130:**1005-1013.

11. Boekschoten MV, Engberink MF, Katan MB, Schouten EG: **Reproducibility of the serum lipid response to coffee oil in healthy volunteers.** *Nutr J* 2003, **2(8):**.
12. Urgert R, Meyboom S, Kuilman M, Rexwinkel H, Vissers MN, Klerk M, Katan MB: **Comparison of effect of cafetiere and filtered coffee on serum concentrations of liver aminotransferases and lipids: six month randomised controlled trial.** *BMJ* 1996, **313:**1362-1366.
13. Casiglia E, Spolaore P, Ginocchio G, Ambrosio GB: **Unexpected effects of coffee consumption on liver enzymes.** *Eur J Epidemiol* 1993, **9:**293-297.
14. Arnesen E, Huseby NE, Brenn T, Try K: **The Tromsø Heart Study: distribution of, and determinants for, gamma-glutamyltransferase in a free-living population.** *Scand J Clin Lab Invest* 1986, **46:**63-70.
15. Nilssen O, Forde OH, Brenn T: **The Tromsø Study. Distribution and population determinants of gamma-glutamyltransferase.** *Am J Epidemiol* 1990, **132:**318-326.
16. La Vecchia C, Levi F, Lucchini F, Franceschi S, Negri E: **Worldwide patterns and trends in mortality from liver cirrhosis, 1955 to 1990.** *Ann Epidemiol* 1994, **4:**480-486.
17. Lam LKT, Sparnins VL, Wattenberg LW: **Isolation and identification of kahweol palmitate and cafestol palmitate as active constituents of green coffee beans that enhance glutathione S-transferase activity in the mouse.** *Cancer Res* 1982, **42:**1193-1198.
18. Lam LKT, Sparnins VL, Wattenberg LW: **Effects of derivatives of kahweol and cafestol on the activity of glutathione S-transferase in mice.** *J Med Chem* 1987, **30:**1399-1403.
19. Schilter B, Perrin I, Cavin C, Huggett AC: **Placental glutathione S-transferase (GST-P) induction as a potential mechanism for the anti-carcinogenic effect of the coffee-specific components cafestol and kahweol.** *Carcinogenesis* 1996, **17:**2377-2384.
20. Cavin C, Holzhaeuser D, Scharf G, Constable A, Huber WW, Schilter B: **Cafestol and kahweol, two coffee specific diterpenes with anticarcinogenic activity.** *Food Chem Toxicol* 2002, **40:**1155-1163.
21. Cavin C, Mace K, Offord EA, Schilter B: **Protective effects of coffee diterpenes against aflatoxin B1-induced genotoxicity: mechanisms in rat and human cells.** *Food Chem Toxicol* 2001, **39:**549-556.
22. Nishi M, Ohba S, Hirata K, Miyake H: **Dose-response relationship between coffee and the risk of pancreas cancer.** *Jpn J Clin Oncol* 1996, **26:**42-48.
23. Giovannucci E: **Meta-analysis of coffee consumption and risk of colorectal cancer.** *Am J Epidemiol* 1998, **147:**1043-1052.
24. Inoue M, Tajiama K, Hirose K, Hamajiama N, Takezaki T, Kuroishi T, Tominaga S: **Tea and coffee consumption and the risk of digestive tract cancers: data from a comparative case-referent study in Japan.** *Cancer Causes Control* 1998, **9:**209-216.
25. Mensink RP, Lebrink WJ, Lobbezoo IE, Weusten-Van der Wouw MP, Zock PL, Katan MB: **Diterpene composition of oils from Arabica and Robusta coffee beans and their effects on serum lipids in man.** *J Intern Med* 1995, **237:**543-550.