

## **Author's response to reviews**

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

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PDF covering letter

Dear Sir,

We thank you and the reviewer for your thorough appraisal of our manuscript entitled: "Coffee bean extracts rich and poor in kahweol both give rise to elevation of liver enzymes in healthy volunteers" (nr 8430822233595650). Your comments are discussed below point by point.

*This is an interesting article but it needs to be re written.*

*The hypothesis being addressed as actually "Robusta coffee oil is associated with increased liver function tests"*

*The data support this hypothesis to a degree.*

A number of studies showed that coffee oil raises levels of ALAT and to a lesser extent ASAT. Two studies suggested that kahweol was mainly responsible for the effect on liver enzyme levels. There are two main types of coffee oil Arabica and Robusta oil. Both contain cafestol and Arabica oil also contains kahweol, whereas Robusta oil only contains minute amounts of kahweol. On the basis of the suggestion that kahweol is the component of coffee oil that is mainly responsible for the effect on liver enzyme levels, we hypothesized that Robusta oil would induce no or small response of liver enzymes while maintaining its cholesterol-raising effect. In the present study we show that the effect of Robusta oil on liver enzymes is similar to that of Arabica oil.

We rephrased the last section of the Background section: "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 Arabica oil, while maintaining its cholesterol-raising effect. In order to test this hypothesis we designed a study in which healthy volunteers consumed Robusta oil." (page 5, line 8 to 13)

*The authors need to be clear that:*

*These changes in LFT may or may not be clinically relevant*

We have added a stronger statement on this point: "At present we have no evidence that the changes in liver enzyme levels induced by coffee oil or unfiltered coffee are of clinical relevance." (page 13, line 23 to 25)

*Ascertain whether those with changes in LFT consume more alcohol*

As mentioned in the discussion section we did not observe a correlation between alcohol intake and the response of liver enzymes. In the present study three subjects showed a large response of liver enzymes. With only three subjects it is not possible to draw conclusions on alcohol intake and the response. However, our previous study with Arabica oil (Boekschoten et al, 2003, Nutr J), in which 13 subjects showed such a large response, we also found no correlation between alcohol intake and the liver enzyme response. In that study non-drinkers were present among the subjects that showed a large response and subjects consuming considerable amounts of alcohol (40 glass/week) did not show such a response to Arabica oil. Therefore we have added the following statement to the discussion: "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,  $\gamma$ GT is decreased rather than increased during consumption of coffee oil, whereas alcohol causes increases in  $\gamma$ GT levels." (page 15, line 9 to 14)

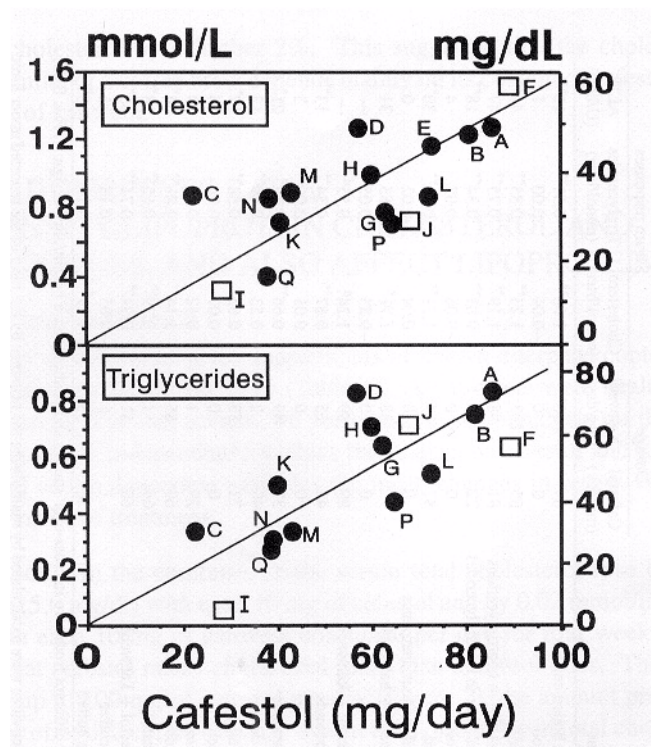
*Explain they have NO evidence that liver damage occurred since no liver biopsies were performed*  
*Cite the presence or absence of data from animals that allude to the point directly above*

We added: "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." (page 13, line 25 to page 14, line 3)

We are aware of unpublished data showing liver pathology in mice after cafestol-feeding, however these data has not been published in the open literature.

*I strongly disagree with the sentiment and statement that "coffee oil potently raises serum cholesterol". The bulk of the data support my opinion. In some people, coffee oil may increase serum cholesterol and in general this is to a small degree".*

We respectfully disagree with the reviewer on this point. His statement may apply to filtered coffee. However we are not aware of any study that failed to show an effect of coffee oil on serum cholesterol. (Heckers, 1994, J Intern Med; Weusten-van der Wouw et al, 1994, J Lipid Res; van Rooij et al, 1995, Am J Clin Nutr; Mensink et al, 1995, J Intern Med; Review in Urgert et al, 1997, Annu Rev Nutr) Only coffee oil that is stripped of cafestol and kahweol has no effect on serum cholesterol. Furthermore, in our opinion the effect of coffee oil or unfiltered coffee is considerable. A meta-analysis of eleven intervention studies showed that each 10 mg of cafestol present in coffee oil or unfiltered coffee raises serum cholesterol by 0.13 mmol/l after four weeks. Please see the figure from Urgert et al, 1997 Annu Rev Nutr below for observed effects of coffee oil and pure cafestol on serum cholesterol (the dose is expressed in terms of mg/day of cafestol but actually most studies were done with coffee oil). One cup of Scandinavian boiled coffee contains 6 mg of cafestol. Consumption of 6 cups of this coffee per day leads to an increase in cholesterol of 0.46 mmol/l or 17.8 mg/dL. This corresponds to the effect of increasing saturated fat intake in the diet by 15-20% of total energy intake. Such a dose is present in about 2 ml of Robusta coffee oil or 1 ml of Arabica oil. In addition, a switch in consumption from boiled to filtered coffee in Finland was associated with a decrease in total serum cholesterol of 0.3 mmol/l (Pietinen et al, 1996, Prev Med). This indicates that cafestol has a considerable effect on health. To our knowledge cafestol is the most potent cholesterol-raising compound identified in the human diet. Therefore in our opinion coffee oil, which contains cafestol, potently raises serum cholesterol.



Relation of daily cafestol intake with mean changes in total cholesterol (upper panel) and triglycerides (lower panel) across 11 short-term experiments carried out by the Wageningen group (circles) and others (squares).: A, coffee oil; B, coffee oil enriched in nontriglyceride coffee lipids; C, coffee oil depleted in nontriglyceride coffee lipids; D, coffee oil; E, mixture of pure diterpene esters; F, mixture of pure diterpene alcohols; G, pure cafestol esters; H, pure cafestol plus kahweol esters; I, Robusta oil; J, Arabica oil; K, Robusta oil; L, Arabica oil; M and N, coffee grounds; P, lipid-rich extract from boiled coffee; Q, Cafetiere coffee. Least-square best-fit equations were:  $\Delta\text{cholesterol (mmol/l)} = 0.015 \times \text{cafestol (mg/day)}$ , and  $\Delta\text{triglycerides (mmol/l)} = 0.009 \times \text{cafestol (mg/day)}$

*It is very important for us to know whether all the blood samples from this study (day 0 and 16 and follow-up) were run as a single a batch. If they were not, the data set may be flawed and it may not be possible to interpret the data and address the hypothesis.*

Due to ethical considerations we were not able to analyze samples in the same run. We had to analyze samples within 24 hours of drawing blood so as to be able to immediately stop treatment in subjects that showed a response of liver enzyme above the predetermined boundaries.

Measurements were performed at a rigorously standardized laboratory. Control reference samples were used within every run in order to ensure that obtained values are comparable to those measured in different runs.

While the analysis of samples in different runs introduces a small source of variation, it is unlikely that this can explain the severe rise in ALAT and ASAT levels in 3 out of 16 subjects. The responses of those subjects were: “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.” (page 10, line 8 to 13) the upper limits of normal were 45 IU/l for ALAT and 50 IU/l for ASAT. Values returned to baseline after treatment was stopped, which also argues against an effect of baseline drift. On the basis of these results we can reject our hypothesis that Robusta oil has no or a small effect on liver enzyme levels and we can conclude that the observed response is comparable to that to Arabica oil.

*I may have missed this - were subjects instructed not to drink coffee and/or alcohol throughout the study?*

Subjects were instructed to maintain their lifestyle and dietary habits but to avoid the use of unfiltered coffee, because unfiltered coffee contains the cholesterol-raising diterpenes cafestol and kahweol. We have now added to the Methods section: “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.” (page 7, line 20 to 23)

The following statement has also been added:

“...no consumption of unfiltered coffee...” (page 6, line 13)

*Do we know that this coffee extract lacked kahweol (ie did the authors measure it)*

Yes, we did and the extract contained a negligible amount of kahweol. This is mentioned in the methods section: “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).” (page 7, line 7 to 9)

*GGT data needs to be presented*

GGT data were presented in Table 2 of the manuscript. Did the reviewer receive Table 2?

*I did not receive figures - there should be a figure that shows ALT for each person before, during and after coffee oil consumption (joined lines). A second graph should show this for AST. The points should be numbered for each subject so that a reader could see if subject 3 responded similarly for ast as alt.*

Such a figure was present in the submitted manuscript for ALAT. Apparently the reviewer never received this figure. We have now also added a similar figure for ASAT.

*No information on dietary intake and weight stability.*

We have no further information on dietary intake and weight stability. However, we think differences in dietary intake (other than alcohol) or weight are not likely to affect the main outcome of this study. Although we did not monitor weight during the study it is unlikely large changes in body weight occurred during the 16 days the study lasted.

*Hepatitis status should be described*

Subjects were questioned about a history of liver disease as part of the eligibility criteria, and all participants denied having had hepatitis. This is now stated explicitly in the Methods section: "...no history of gastrointestinal or liver disease..." (Page 6, line 13 to 14)

*There is no control group - the authors should acknowledge this*

We added: "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" (page 15, line 2 to 5)

*Minor: The paper is difficult to read. The study design is different to the data presentation in the results In future I would recommend double spaced manuscripts.*

We added subheadings to the Results section to make the reading more convenient:

**"Response of liver function parameters to Robusta oil"** (page 10, line 18)

**"Serum lipid response to Robusta oil"**(page 11, line 13)

The manuscript is now double-spaced, we also added line numbers.

We hope that this revision will prove to the required improvement and look forward to your decision.

Sincerely yours,

Mark Boekschoten