Author's response to reviews

Title: The effects of plant stanol ester consumption on arterial stiffness and endothelial function in adults. A randomised controlled clinical trial.

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Author's response to reviews: see over
Point-by-point responses to Reviewer 1

Thank you for the comments and constructive criticism, which have improved the manuscript. Please find our responses below. The corrections in the manuscript are highlighted using red font. A native English colleague has edited the language of the manuscript.

Major Comments

1. **It is rather surprising that authors found a deterioration of CAVI and AI in controls, which contributed to the statistical significance of differences with the respective variables of the treatment group, in a relatively short period of time (6 months).** For example, CAVI in the control group (healthy people without diabetes, arterial hypertension or dyslipidemia) was 8.66 and was increased to 8.73, when authors state that "CAVI #8 reflects increased arterial stiffness, and if CAVI #9, arterial stiffness is significantly increased [20]." If CAVI in healthy people increases by 0.22 within 6 months, in a 6 year period its value would be > 10 (extremely high for anyone, especially healthy people). There are two other issues with CAVI. First this tended to increase more in male controls and if men were as many as women, than there might have had an even greater increase in the control group!! The second issue is that CAVI is not considered to be related with hypercholesterolemia (Soska V, Dobsak P, Dusek L, et al. Cardio-ankle vascular index in heterozygous familial hypercholesterolemia. J Atheroscler Thromb. 2012;19(5):453-61. Epub 2012 Jan 12.) but it is mainly related to smoking (Soska V, Frantisova M, Dobsak P, et al. Cardio-Ankle Vascular Index in Subjects with Dyslipidaemia and Other Cardiovascular Risk Factors. J Atheroscler Thromb. 2013 Mar 4. [Epub ahead of print] with a value of 7.8 for heavy smokers). Thus, it might not be such a good measure for arterial stiffness in patients with dyslipidaemia. On the other hand the increase of AI in the control group from 8.2 ± 2.5 to 11.5 ± 2.7 (by 3.3 or 40.2%) is extremely high for a 6 month period for anyone, especially healthy people. Probably this is the reason that changes in AI in Figure 2, second panel, is reported in absolute units, while the rest of the variables (without any exception) are reported as % change.

Response: The controls were not ‘healthy’; they were controls only to plant stanol ester consumption, but similarly to the plant stanol ester group, about two thirds of them were hypercholesterolemic, about every seventh had hypertension, and they were middle-aged, so that even though they did not have symptomatic cardiovascular diseases, Systematic Cardiovascular Risk Estimation (SCORE) indicated moderate or high risk in 65% of the subjects. In the review article by Shirai et al (ref 13) CAVI increased with age, and was increased by eg. dyslipidemia, hypertension, smoking, metabolic syndrome, and diabetes. On the other hand, CAVI values could be reduced with hypertension control, blood glucose control, with lipid lowering agents, and stopping to smoke. Accordingly, it can be anticipated that there was increased arterial stiffness in the whole study population at baseline.

Second, in our study population CAVI was correlated with serum total and LDL cholesterol and serum triglyceride concentrations at baseline (Results) suggesting that serum cholesterol levels were among those factors affecting arterial stiffness. In the review by Shirai et al (ref 13) dyslipidemia is one of the factors related to increased CAVI, and dyslipidemic treatment can improve CAVI. Regarding whether the 6-month time period is too short to affect CAVI, in ref # 28 and 30, the length of the interventions were 3 and 6 months, and there were significant changes in CAVI. In addition, the large hard endpoint- statin trials have demonstrated that the treatment
groups were diverging from the control groups after one year with significant reductions in hard end points. Along these lines we think that it is no wonder that during the 6 month period without hypocholesterolemic treatment in the control group the arterial stiffness was still impairing. Methodologically the reproducibility of these measurements (CAVI, AI, and RHI) is good as mentioned in Methods, so that methodological aspects hardly can explain the results. Longer follow up period with repeated measurements with appropriate intervals, such as 3 months, would reveal more clearly the dynamic changes in diet related interventions of normal daily living.

Third, regarding AI measured with EndoPAT, the unit of the variable even at baseline is given as percentage. A note added to Methods, vascular variables, para 4.

2. It would be helpful for the reader to report the mean aortic PWV data in Table1.

Response:
Aortic pulse wave velocity would be very interesting and may improve the interpretation of indirect assessment of arterial stiffness. Aortic pulse wave velocity, however, was not calculated from the signals of any measured variable. We used only the standard variables produced by the algorithms of the device.

3. Discussion: "only few studies have evaluated the effect of LDL cholesterol lowering on AI."

Response:
We have corrected the sentences in Discussion, p 15, para 2, and added the references 1-3 (#33-35) mentioned by the referee and underlined the evaluation of the differences in techniques to assess AI.
Point-by-point responses to Reviewer 2.

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Major compulsory revisions

1. Abstract: Conclusions: Since RHI did not significantly change in the study, it may not be right to say that “the more cholesterol was reduced, the more RHI was increased”, it can be said that the parameters are correlated however.

Response: The text indicated has been deleted.

2. Methods: Study Design, paragraph 2: Can you please describe the study protocol in more detail: please add when the subjects came to the research center and when they were contacted via telephone. Please describe what was done during each visit? How many times blood was sampled, when were vascular measurements done. Was this only at the beginning and end? This is not clear now. What was asked during telephone contact?

Response: The Study Design para 2 has been rewritten and hopefully is now more detailed.

3. Methods: Vascular Measurements: Paragraph 1: reference 13 for method of PWV measurement: this article does not thoroughly describe the measurement. In addition, reference 21: this does not describe CAVI as an indicator for lifestyle modification, please put the correct references.

Response: Ref 13 is demonstrating in detail the methods and calculations regarding CAVI, so that we feel that ref 13 is a decent one here. Ref 21 (now 22) has been changed.


Response: The paragraph (now para 3) has been rewritten to give better description of these markers. PAT is the method of measuring peripheral pulse volume amplitude, while RHI and AI are markers of endothelial function and arterial stiffness derived from this measurement.

5. Results: Vascular Variables: Since a gender difference has been observed here, it might be interesting to see whether this gender difference also exists for serum lipids and lipoproteins? Especially since CAVI was correlated with serum total and LDL cholesterol values. In addition it is interesting to see in table 1 that although not significant the CAVI in women tends to increase in the stanol
**Minor Essential Revisions**

1. **Background**: consuming food products added with plant stanol ester, change to with added plant stanol ester

Response: Corrected as suggested.
2. Background: reduced by 9% at the 2g/day dose of plant stanols, change to reduced by 9% at an intake of 2g plant stanols/day

Response: Corrected

3. Methods: Study Design: 2nd paragraph: in the end of the study, change to at the end of the study

Response: Corrected

4. Methods: Diet: a theoretical daily amount, change amount into intake

Response: Corrected

5. Methods: Diet: 3-d food record kept at baseline and in the end of the study, change in into at

Response: Corrected

6. Methods: vascular measurements: 1st paragraph: After10 minutes, please put space between after and 10

Response: corrected

7. Discussion: paragraph 4: lifestyle habits were kept as unchanged as possible, change to lifestyle habits were kept unchanged as much as possible

Response: Corrected

Discretionary Revisions

1. Abstract: Methods: I would change symptomless subjects to apparently healthy or asymptomatic subjects

Response: Changed

2. Methods: laboratory Methods and Measurements: Laboratory measurements, taken to ensure normal health: please define what parameters. Where they unchanged during the study?

Response: The laboratory parameters are listed in Laboratory Methods and measurements. None of them were changed during the intervention, a note added to Results, Intervention, first para.

3. General: in the methods section is mentioned that CAVI is considered to be independent of blood pressure; however this study showed a correlation between
systolic blood pressure and CAVI, do you have an explanation for this?

Response: The sentence in methods is misleading and it should be: CAVI is considered to be independent of blood pressure at the time of measurement. Corrected in the text and a new reference, #18, is added.

4. General: Since the subject group is rather diverse in BMI, lipid parameters, medication and background disease I am still wondering if this may have influenced the results. Could you provide a table where this background is described per group?

Response: The groups did not differ from each other regarding BMI, lipids, medications and background diseases as mentioned in the Results section. However, a new Table, Table 2 has been added illustrating these variables in the two groups. Because of the Table, the text in Results, baseline characteristics, para 2, has been modified.
Point-by-point responses to Reviewer 3.

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**Major:**

*Introduction:* Please acknowledge the fact that the studied outcomes are also surrogate indicators for cardiovascular diseases and comment on whether they are valid surrogate indicators or not.

Response: These important points have been added to Introduction.

*Method:*

- Please add information about the time and frequency of consumption of plant stanol products

Response: The information is added to Study design

- Please provide more details about the method of randomization and who was blinded and how

Response: The information is added to Study Design.

- How did you measure the level of physical activity?

Response: We used a structured questionnaire, in which frequencies of fitness or functional exercise were asked. The same questionnaire has been used in our studies for several years.

- The sample size should be calculated based on the primary outcome of interest which is here not LDL.

Response: When planning our study in 2010 we had plenty of information on the LDL cholesterol lowering by 10% with added plant stanol intake of 2-3 g/d. However, we could not find respective relevant information of dietary interventions on CAVI, RHI or AI. Accordingly, we wanted to find out the responses of CAVI, RHI and AI when LDL cholesterol was lowered by 10%. This is the reason why we had to choose to make the power calculations using the information we had on LDL cholesterol level.

- Do a subgroup analysis for the cardio-ankle vascular index in large and as augmentation index in peripheral arteries, and endothelial function as reactive
hyperemia index by responding or not to plant stanol therapy based on changes in LDL.

Response: We did these subgroup analyses, but the results were not different from the whole groups.

Discussion:
- Comment on the clinical significance of the observed reduction in CAVI.

Response: CAVI is a novel technique to assess arterial stiffness. Although there exist large scale promising results from the use of CAVI in health of some ethnic populations, further studies are needed to evaluate its usefulness in different populations and compared to other recommended methods, such as carotid-femoral pulse wave velocity (van Bortel LM et al, J Hypertens 2012;30:445-8). This is added to Discussion, and a new ref, # 31, is added.