Author's response to reviews

Title: Can red yeast rice and olive extract improve lipid profile and cardiovascular risk in metabolic syndrome? A double blind, placebo controlled randomized trial.

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Author's response to reviews: see over
Dear Editor,

Thank you for giving us the opportunity to revise our manuscript entitled

“Can red yeast rice and olive extract improve lipid profile and cardiovascular risk in metabolic syndrome? A double blind, placebo controlled randomized trial.”

Some of the suggestions were particularly helpful to make our paper and analysis more clear; below you will find a point-by-point reply to all remarks and suggestions. In the manuscript, all changes are marked in red.

Furthermore, we had the manuscript copy-edited to improve the English language. Changes made by the copy editor are in green.

Remarks of Editor

Authors should address the concerns raised by the reviewers carefully. In addition to those concerns, I would like the authors to give more thoughts on their statistical analysis. I agree with one of the reviewers that comparison between intervention group and control group needs to be clearly described. Although table 3 appears having compared the before-after difference between the two groups, no description was given in results.

Thank you for this remark. After re-reading the manuscript, we agree that the statistical analysis was not clearly described which led to confusion. All p-values in table 3 and 4 refer to a comparison of the effect (= the before-after difference) between intervention and control group. This is evidently the standard analysis for RCTs (which we also used in our former article on the subject in this journal, Verhoeven V et al, BMC Complement Altern Med. 2013 Jul 18;13:178).

The comparison was mentioned in the tables but nowhere clearly stated. We changed this in abstract methods section and we added the values of the control group in the text of the results section to make it more obvious.

In addition, the % in table 3 was not clearly noted.

We tried to make this more clear in the table.

Moreover, as authors used multivariate linear regression, why were pretest and group membership not included in the model to provide a better comparison between intervention and control? ANCOVA may also be an appropriate approach. More details on why and how linear regression was performed are needed (e.g. Describe dependent variables and independent variables.)

We used univariate analysis to describe the outcome parameters of the study (which we believe is appropriate in studies with randomization). The multivariate analysis was used only to explore the data further, more specifically to check effect modification within the intervention group, i.e. to
check whether the observed effect (in the intervention group only) was influenced by confounders such as age or gender. We described this more clearly in the methods section and in the results section.

The purpose of the correlation analysis is not very clear. Correlation between which outcomes and the rationale for this analysis need to be discussed. The correction analysis results in this paper makes one wonder the necessity of the analysis, as nothing is unexpected. Again, why is this analysis important for authors?

We agree that this secondary analysis is not absolutely necessary. The correlations between changes in biochemical parameters were indeed not unexpected. We mainly wanted to check if the (unexpected) effect on blood pressure was correlated with the effect on the lipid profile (whether the subjects with the greatest LDL reduction had also the greatest effect on their blood pressure). However, the correlation we found is only weak and the sample size calculation did not take into account this extra analysis; therefore we suggest to take this part out.

Reviewer 1

The paper entitled: Can red yeast rice and olive extract improve lipid profile and cardiovascular risk in metabolic syndrome? A double blind, placebo controlled randomized trial written by Veronique Verhoeven et al. examines the effect of a nutritional supplement combining red yeast rice and olive oil extract on the lipid profile and on oxidative stress in a population of patients with Metabolic Syndrome. This article provides interesting aspects about managing the cardiovascular risk factors in Metabolic Syndrome patients. Although the primary outcome measures were the before-after difference in lipid levels and oxidized LDL as a parameter of oxidative stress, the authors need provided the comparison between results of intervention group to control group.

We fully agree with this (see our answer to the first remark). The results which are presented do compare both groups, although it was not clearly described in the paper. Sorry for the misunderstanding! We changed this in the abstract, methods and results section.

Moreover, in Abstract, line 32, it is stated: Statins are the drugs of choice to treat dyslipidemia, but whether they should be used for primary prevention in all MetS patients is not yet clear. In our opinion, today from international guidelines, the use of supplement food as the product used in the present article can be consumed into lifestyle changes. As an example, an international panel of the International Atherosclerosis Society has developed a new set of recommendations for the management of dyslipidemia. For primary prevention, the recommendations emphasize lifestyle therapies to reduce atherogenic lipoproteins; drug therapy is reserved for subjects at greater risk (Expert Dyslipidemia Panel of the International Atherosclerosis Society Panel members. An International Atherosclerosis Society Position Paper: global recommendations for the management of dyslipidemia--full report. J Clin Lipidol 2014;8(1):29-60.
We agree with this remark, moreover we had already stated in the paper “Whereas statin treatment effectively reduces CVD in secondary prevention and being standard therapy for primary prevention in diabetes, there is no consensus in favor of a generalized use for primary prevention in the heterogeneous group of patients with MetS.” (r 75-77). We changed the phrase in the abstract to “Lifestyle changes are the cornerstone of MetS treatment; statins are the drugs of choice to treat dyslipidemia in higher risk patients, but they are not recommended for primary prevention in all MetS patients.”. Furthermore we added the suggested reference in the introduction (r75, reference 7).

The results from effect of product on cardiovascular risk in the participants are not described.

We described them in the results section under the heading “Cardiovascular risk”:

The cardiovascular risk according to SCORE (10-year risk of fatal CVD) ranged from 1 to 18% in the intervention group and from 1 to 46% in the control group (not normally distributed, median: 2% in both groups). The risk score changed after the intervention in 8/26 subjects in the intervention group (4 times -1%, twice -1.5%, once -4%, once -7%), mainly through the lowering of systolic blood pressure. In the control group, the risk decreased with 2% in one participant, and it increased in two people, respectively with 1 and 3%.

In abstract, information about the product consumed should be provided.

We added the following statement to the abstract: “The study product contained 10.82mg of monacolins (of which 5.88mg of lovastatin) and 9.32mg of hydroxytyrosol per capsule, and is commercialized as Cholesfytol plus.”

Moreover, diet consumed during the study is a key point in this type of trials.

We assessed the diet of our study participants at the beginning of the study (in the context of a longer cohort study), but not during the study. We imposed no diet on the study participants during the study (we added a statement in the manuscript).

Thus we don’t know whether the diet changed during the study, but we provided some extra (cross-sectional) information in table 1, which shows that intervention and control group did not significantly differ in some key dietary habits at the beginning of the study (vegetarian lifestyle, meat consumption, alcohol consumption).

Reviewer 2

1. There is confusion in the definition of sample size. In the abstract and in the manuscript (methods section) authors defined as primary outcome the differences in lipid levels (generally and not in particular LDL cholesterol) and in oxLDL. They statement that sample size was calculated to establish a difference in LDL cholesterol of 15%. Primary outcome must be clearly defined and should be the before-after difference in LDL cholesterol.
We agree with this remark. We described better the primary outcome parameter on which the sample size calculation was based, in the methods section “A sample size calculation based on SD (standard deviation) values in previous studies, determined that 40 patients were needed to establish a difference in LDL-cholesterol reduction of 15% between intervention and control group (power 0.80, significance level 0.05).” We changed this as well in the abstract section:

“Primary outcome measure was the difference in LDL reduction between intervention and control group.”

2. In methods section the authors do not describe any of the methodologies used to measure the parameters analyzed. However, it is essential to describe these methodologies, especially as regards the oxLDL, since this is one of the primary outcomes and there are many different assay kits to measure them.

We agree with this, especially the remark on oxLDL. We added a brief description on the methodologies used and a more detailed description for oxLDL in the methods section:

“Analyses were performed in a certified commercial clinical laboratory (Laboratory of Molecular and Clinical Pathology (RIATOL), AML, Sonic Healthcare Benelux, Emiel Vloorsstraat 9, 2020 Antwerp, Belgium); in accordance with routine practice CH, HDL, TG, CK and glucose were measured by means of spectrophotometry; apoA1 and apoB by means of nephelometry, and HbA1c by means of high pressure liquid chromatography. Plasma oxLDL was analyzed by an enzyme immunoassay kit (Mercodia OxLDL sandwich ELISA kit, Mercodia AB, Uppsala, Sweden). Mercodia OxLDL ELISA is a solid phase two-site enzyme immunoassay, in which two monoclonal antibodies are directed against separate antigenic determinants on the oxidized apolipoprotein B molecule. OxLDL in the plasma sample reacts with anti-oxidized LDL antibodies bound to microtitration wells (mAb 4E6) and horse radish peroxidase (HRP) conjugated anti-oxidized LDL antibodies in the solution. The oxidation of 3,3',5,5'-tetramethylbenzidine (TMB) by HRP is measured spectrophotometrically at 450 nm.”

3. Authors define the oxLDL as parameter of oxidative stress. This statement is not completely correct. OxLDL are in fact an indirect parameter of oxidation, the parameter of lipid peroxidation is represented by malondialdehyde.

We agree that it is more correct to define oxLDL as an indirect biomarker of oxidative stress and rephrased this in the methods section. Furthermore we added a reference of EFSA, which accepts the measurement of oxidized LDL particles as a valid in vivo measurement of oxidative damage to lipids (reference 18).

The altered text (methods section): “Furthermore, we measured oxidized LDL (oxLDL); OxLDL represents a mixture of oxidatively modified LDL particles, containing oxidatively modified lipid and apoB protein components. Although an indirect biomarker of oxidative stress, assessment of the oxidative modification of LDL is relevant given its pro-atherogenic properties (16- 18).”
4. In methods section, authors stated that they evaluated with questionnaires all the factors that can affect lipid levels and oxidative stress. No data about these questionnaire were shown. It is important, however, that these data are shown to attribute all the results only to therapy studied because it is well known that diet, for example, greatly influences both the levels of LDL cholesterol (up to 20%) and all the parameter of oxidative stress.

This is true. We evaluated diet and lifestyle factors at the beginning of the study – in the context off a longer cohort study. However, we have no data on changes of these parameters during the study. No diet was imposed on the study participants during the study (this is stated in the manuscript).

We added some factors which may affect lipids and oxidative stress to table one, which shows that intervention and control group did not differ in some key dietary habits and lifestyle factors at the beginning of the study, more specifically:

- vegetarian lifestyle
- meat consumption >5x/week
- daily alcohol consumption
- menopausal status in women
- smoking
- level of perceived stress

We hope this is sufficient information.

In table 2 the unit of measurement of HbA1c must be changed from mg/dl to mmol/mol.

Thank you, this was evidently an error – it was changed.

As regards secondary outcomes, it’s better to show in table or in the text all the value of CPK. Authors stated only that a mild CPK elevation was observed in all subjects.

We added the mean values with their standard deviations and the range for both the intervention and the control group. Furthermore we added the cut off values of our laboratory.

As regards the analysis of the content of the capsules, authors should better specify the content of monacolin K and lovastatin separately.

We added this information in the abstract and in the methods section.

Authors should discussed the significant before-after differences in term of TG and apoB obtained in the intervention group.

We added a paragraph in the discussion section.
An article of insufficient interest to warrant publication in a scientific/medical journal
We do not agree with this statement (I would say obviously). Food supplements containing red yeast rice have been marketed aggressively in the past years as a natural way to lower cholesterol. However, the large majority of commercially available products (including combinations of active ingredients like this product) have not been studied according to current research standards. There is a marked variability between products with respect to monacolin levels per gram of labeled “active” product, and there is a lack of quality control for food supplements. We strongly believe that every new food supplement claiming health effects should be properly (and independently) characterized studied for efficacy and safety, and that study results should be made accessible to health care providers and patients.

We hope we have clarified our manuscript and answered in a satisfactory way to the remarks and suggestions of the Editor and Reviewers.
Please let us know if you need any further clarification.

Best regards,
Veronique Verhoeven,
on behalf of all co-authors