Author's response to reviews

Title: Postprandial glucose-lowering effects of fermented red ginseng in subjects with impaired fasting glucose or type 2 diabetes: A randomized, double-blind, placebo-controlled clinical trial

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Author's response to reviews: see over
Reviewer: Vladimir Vuksan
Reviewer's report:

The manuscript by Mi-Ra Oh entitled “Postprandial glucose-lowering effects of fermented red ginseng in subjects with impaired fasting glucose or type 2 diabetes: A randomized, double-blind, placebo-controlled clinical trial” provides very interesting evidence on the possible therapeutic domains of ginseng derivatives. However, there are several important concerns that need to be addressed:

Major:
1) The responses to previous review were not provided. I could not find the ‘point-by-point’ responses in the revised manuscript. This would have been highly helpful in re-review.

   The following are the previous point-by-point responses in the revised manuscript.

First comments to the Author
► Please provide details in your methods section on how the sample sizes necessary to ensure the statistical significance of your study were calculated.

   The sample size was statistically determined to obtain a power of 80% with an alpha of 0.05. In order to demonstrate effects in 2-h postprandial glucose level, which was calculated to be a 0.4 mmol/l reduction with a standard deviation of 0.53 mmol/l, a sample size of 32 (16 in the FRG group and 16 in the placebo group) was required. Assuming a 20% loss to follow-up, 42 participants were selected. The two groups were equal in size in order to obtain the greatest statistical power.

► We note from your Acknowledgements section that Wonkwang Pharmaceutical Co., Ltd provided both financial support and fermented red ginseng for your study. Please note that we would consider this to constitute a potential competing interest, and would therefore ask you to include this information in your Competing Interests section. Please also confirm whether Wonkwang Pharmaceutical Co., Ltd played any part in the design, implementation or interpretation of your study. If so then this should also be declared. If not then, for the sake of clarity, we would still ask you to include a statement to this effect in your Competing Interests section.

   We revised this point in the competing interests section (on page 12-13) as follows: Wonkwang Pharmaceutical Co., Ltd. gave us permission to use their commercially available FRG product for our study. Furthermore, they provided the intervention products and financial support for the completion of the study. Wonkwang Pharmaceutical Co., Ltd. had no further methodological or any other input in design, execution, or reporting of this study. Wonkwang Pharmaceutical Co., Ltd. had no access to the study protocol, names of participating physicians, or raw study data. Before starting the study, it was agreed specifically that the study would be published regardless of the results.

Second comments to the Author
► I have reviewed the manuscript briefly and there are a few concerns. The authors state that they have performed an intention to treat analysis using all data. But, it would appear that they lost 4 people in the follow up of the intervention group and one in the control group. How did they perform the analysis with everyone then? Something about a form of imputing data should be provided and a comment about how that imputation might have affected the
results should be provided in the discussion. I would also find it helpful to have some idea of whether these findings are clinically important or not.

We revised this point in the methods section (on page 6) as follows:

Statistical analyses were performed using SAS version 9.2 for Windows (SAS Institute, Cary, NC, USA). For statistics, we used a mixed-effect model approach for intention-to-treat analysis with missing values [20]. Data are shown as the mean values and the standard error of the mean (SEM).

Third comments to the Author

► We contacted you recently to request adjustments to your submission, prior to review. We hope that the email below reached you safely. In order to facilitate prompt peer review, a complete and structured submission is required. We hope that you will be able to upload your revised files in the next 4 days. Please note that non-adherence to the journal’s ethical or formatting policies may prevent or delay publication of your manuscript should it be found to be acceptable. We look forward to receiving your updated manuscript soon, in order to proceed promptly with review.

We stated the name of the ethics committee and the reference number in the methods participants section (on page 4) as follows: The study, which was conducted according to the Declaration of Helsinki, was approved by the Functional Food Institutional Review Board (FFIRB) of Chonbuk National University Hospital (WKP-FG7070-001).

2) The authors should clearly state what were their primary and secondary analyses?

We described the primary and secondary outcomes in the methods section (on page 6) as follows: All of the presented data are from the intention-to-treat population. The primary outcome (postprandial glucose) and the secondary outcomes (total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides) were used to compare the FRG group to the placebo group.

3) What is the rationale for evaluating changes in lipids? Moreover, this was done in a normolipidemic population which could potentially form a subject bias.

As answered in the above question, the primary outcome for this study was to observe changes in postprandial glucose after consuming fermented red ginseng, which is why we recruited subjects with impaired fasting glucose or diabetes. We checked the changes in lipid profile to assess the possible application of fermented red ginseng in hyperlipidemic patients. For this, of course, we should have recruited hyperlipidemic subjects. Unfortunately, we could not recruit enough subjects with both hyperglycemia and hyperlipidemia. For this reason, the changes in lipid profile became a secondary outcome.

4) Please provide further details on your study population. How many participants were diagnosed with type 2 diabetes? Was insulin therapy included? How many individuals were on prescription antihyperglycemic medication? Did they take the medication during the glucose challenge test?

We described the study population in more detail in the results section (page 7) as follows: Eighty-four participants were assessed for eligibility, and a total of 42 participants (mean age = 53.3±8.4 years, 28 males and 14 females, mean body mass index = 24.9±3.3 kg/m², fasting glucose = 6.5±0.5 mmol/l) met the inclusion criteria. Nineteen participants were diagnosed with type 2 diabetes, and 23 participants had either impaired fasting glucose or impaired
glucose tolerance.

5) What is a rationale for selected dosing given that this is the first human intervention?
   We determined human dosages from our previous animal study (unpublished results). In our animal study, 300 mg/kg of fermented red ginseng (FRG) effectively reduced blood glucose and insulin levels in streptozotocin-induced diabetic rats. We used a conversion factor and accounted for body weight to calculate the human dosage from following equation: 300 mg/kg of FRG in rats × 0.16 (rat conversion factor) × 60 kg in humans = 2,880 mg/day

6) The ginsenoside profile of FRG should be included, according to the Consort statement for reporting of herbal interventions.
   We analyzed the ginsenoside profile of FRG and added the data to Table 1.

Minor
- Please ensure manuscript is proof-read for grammar inconsistencies.
   Our manuscript was proofread by e-World editing located in Seattle.

- Were subjects with fasting plasma glucose >140mg/dL excluded?
   Yes. We only included diabetic patients with glucose levels less than 140 mg/dL. We stated this point in the method section as follows: Subjects age 20-75 years with a fasting glucose of 100-139 mg/dL with at least two follow-up measurements were recruited and included in the study.

- What was the basis of criteria used to calculate power?
   We further described this point in detail in the methods section (page 6) as follows: The sample size was statistically determined to obtain a power of 80% with an alpha of 0.05. In order to demonstrate effects in 2-h postprandial glucose level, which was calculated to be a 0.4 mmol/l reduction with a standard deviation of 0.53 mmol/l, a sample size of 32 (16 in the FRG group and 16 in the placebo group) was required. Assuming a 20% loss to follow-up, 42 participants were selected. The two groups were equal in size in order to obtain the greatest statistical power.

- In the context of this pilot trial it would have been very informative to measure concentration of compound K in participants.
   According to our IRB protocol, we discarded all samples after the clinical trial, so there is no way to analyze compound K at this time.

- What was the available carbohydrate load of the standard meal?
   The standard meal was composed of 76.4 g of total carbohydrate and 6.4 g of fiber. Thus, the available carbohydrate of the standard meal was 70 g. We made this point clear in the methods section as follows: The subjects were asked to consume a standard meal [584.1 kcal, caloric contribution: 52% carbohydrates (containing 70 g of available carbohydrate), 18% protein, and 30% fat] after a 12-h overnight fast.

- Table 2 lists FRG abbreviation as “fasting plasma glucose”. Please ensure this and similar errors are corrected.
   We corrected our mistakes. Thank you for your correction.
Reviewer: Iris F Benzie
Reviewer's report:

Ginseng and glycaemic control; Oh et al
The paper describes the results of a 4 week trial with fermented red ginseng (FRG) on fasting plasma glucose (FPG) and insulin and post-prandial changes in these in 42 (21 on treatment and 21 in placebo) subjects with impaired glucose tolerance of type 2 DM. Lipid profile was also measured in subjects pre and post treatment. Result showed decreased postprandial glucose and insulin; no changes in lipids were seen.

Comments:
The paper is written clearly, though the abbreviations used (FPG, FRG and MTT) could cause some confusion.

We spelled out all abbreviations except FRG and AUC.

In the statistical analysis part (page 6), it is stated that it was determined that 32 subjects were needed in each group to allow for a 20% drop out rate - should this be overall (i.e. 16 in each group)?

We made this point clear in the Methods section (on page 6) as follows: The sample size was statistically determined to obtain a power of 80% with an alpha of 0.05. In order to demonstrate effects in 2-h postprandial glucose level, which was calculated to be a 0.4 mmol/l reduction with a standard deviation of 0.53 mmol/l, a sample size of 32 (16 in the FRG group and 16 in the placebo group) was required. Assuming a 20% loss to follow-up, 42 participants were selected. The two groups were equal in size in order to obtain the greatest statistical power.

The results for glucose and lipids should be given in SI units (mmol/l).

We changed all units as suggested.

Why are data presented as mean ± SE, rather than SEM?

Our original data were represented as the mean ± SEM. We are sorry for our mistake.

It is not clear in table 2 if the difference is post-placebo vs. post treatment, or (as it should be) response to placebo vs. response to the ginseng treat – this needs to be clarified.

We compared the differences between response to placebo and response to FRG. We made this point clear as follows: 1) Analyzed by linear mixed-effect model, and the p-value represents the comparison to the baseline visit. 2) Analyzed by linear mixed-effect model and the p-value represents the comparison to the placebo group.

Several previous studies have shown that ginseng helps modulate post-prandial glycaemic response. It is not clear how this particular study adds much to knowledge about this. The authors should clarify that – and perhaps discuss why they chose to compare response to placebo rather than against a non-fermented ginseng preparation, which would have been more interesting. They state that fermented red ginseng has “more potent pharmacological activities than non-fermented ginseng” - for this statement to be confirmed the comparative treatment would need to be non-fermented ginseng, not placebo.

As the reviewer pointed out, beneficial effects of ginseng or red ginseng on metabolic or immunological disorders have been reported by several groups. Additionally, as we described in the introduction section, glucose-lowering effects of fermented red ginseng were also
reported in an animal study. However, no studies in humans have been reported. Similar to
the animal study, this human study questioned whether fermented red ginseng has glucose-
lowering effects. Most likely, our next study will be a comparative study between fermented
and non-fermented red ginseng. We changed our final conclusion as follows: Further studies
with a larger number of subjects over a longer duration and comparative studies between
fermented red ginseng and non-fermented red ginseng are needed.

Reference 20 is incomplete
   We corrected reference 20.