Author’s response to reviews

Title: Freeze-dried strawberry powder improves lipid profile and lipid peroxidation in women with metabolic syndrome: baseline and post intervention effects

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Version: 2 Date: 8 August 2009

Author’s response to reviews: see over
The Editor
Nutrition Journal
Biomed Central
August 7, 2009

Re: Freeze-dried strawberry powder improves cardiovascular risk factors in obese women with metabolic syndrome: pre- and post intervention effects.

We thank the reviewers for their valuable comments and concerns which have been addressed point wise for each reviewer as follows-

Reviewer: Kalidas K Shetty

1. Is ellagic acid content of FSP known? This would be good to know since it has been linked to what has been observed in the plasma and potential effects of the same.- This information has been included on Table 1.

2. Is 16 subjects sufficient and also period of the study? Is it too short.- yes, based on the power calculation we needed a sample size of greater than 10 which has been included in the text under Statistical analyses. Since, this was a pilot study in humans to test tolerability, safety, and biological effects of freeze-dried strawberry powder, and we used a high dose (~ 500g fresh strawberries), we hypothesized that the effects will be observed within a short period of 4 weeks.

3. 9/16 took supplements? What is the effect of these and how was this normalized to see the strawberry benefits is not clear.- We compared the supplement users versus the non users and found no statistical differences in the parameters of interest in this study. This information has been added in the Result section.

Reviewer: ana Cecilia C marin-guerrero

1. In the title: obese women with metabolic syndrome. The definition of the metabolic syndrome given by the authors, according to National Cholesterol Education Program Adult Panel III treatment, includes obesity, therefore the term (obesity) could be omitted in the title. – this has been revised in the title.

2. The aim of the study is properly raised, but the hypothesis the authors want to test, does not agree with the title of the article. It is important to take into account that the cardiovascular risk factors are more numerous than those mentioned in the hypothesis.- this has been addressed in the section of hypothesis as “selected cardiovascular risk factors including markers of lipid peroxidation and inflammation”.

3. The results: the main results show the significant reduction in total cholesterol and the reduction of lipid peroxidation indicators. With only these two outcomes it can’t be said
that there has been an improvement of cardiovascular risk factors. - this has been revised in the abstract, result and discussion sections. We have deleted the concept of “reducing or improving CVD risk factors” versus improvements in “lipids and lipid peroxidation” or “selected CVD risk factors”.

4. The discussion reads: "The strawberry powder also provided an additional 8g of fiber to the diet which may contribute to overall satiety and possible replacement of unhealthy snacks in the daily diet”. This assumption is contradicted by the said earlier by the authors in the results section, that there were no significant changes in the diet of women in the study. – this has been revised and re-stated as “Furthermore, the total dietary fiber content of the strawberry drink (8 g/day) may contribute to the cholesterol lowering effects”.

5. The conclusions: Again the authors are using the terms metabolic syndrome and obese, when obesity is included in the metabolic syndrome definition. We can’t consider the freeze-dried strawberry powder as a potential therapeutic strategy to reduce cardiovascular risk factors, when in fact this study has only found improvement in LDL cholesterol and some of the markers of oxidative stress.- these have been revised in the conclusion section.

Discretionary Revisions:

1. What kind of compensation has been given to the women who participated in the study?
   – we gave them $30 on a weekly basis
2. As the study population has not been selected at random, can you think about some possible limitation during the analysis of the data? - we thoroughly checked the data for outliers which however did not significantly affect the results.

Reviewer: Diane L McKay

1. The hypothesis and outcome markers of interest are clearly stated under the Methods section (Objective). However, the authors must clearly state up front that this study is uncontrolled, and that comparisons are made between levels observed at baseline vs. those collected at the end of the study. A brief statement of the study goal should also be included in the Background section.- this has been revised in the background and Methods (Objective) sections.
2. If the purpose of this study is to examine the effects of a strawberry drink on selected biomarkers in subjects with metabolic syndrome, it is unclear why the authors emphasize the tolerability and safety of this beverage (in the Conclusion) over its effects on CVD markers.- this has been corrected and “tolerability and safety” have been included in the abstract and the background sections. Conclusion has been revised to highlight effects on selected biomarkers in metabolic syndrome, in addition to effects on safety and tolerability.
3. There are several grammatical errors in this manuscript (too numerous to list individually). There are also several instances where sentence structure is confusing.
For example, in the middle of the first paragraph of the Background section, the sentence beginning with “Keeping in view the critical role of oxidative stress…” is incoherent. Throughout the manuscript, the information is presented in sporadic bursts rather than in a logical progression. That is, there is no logical flow from one sentence to the next. – these have been corrected throughout the manuscript.

4. Information presented in the Background section is overstated. For example, in the Background section (first paragraph) the authors mention a study conducted in rat smooth muscle cells. They suggest the results of this study show a decrease in the process of atherosclerosis. This is incorrect. Rather, the results of the study cited suggest a potential effect on the progression of atherosclerosis. One cannot follow the progression of atherosclerosis in cell culture studies. – this has been corrected in the Background section.

5. Information presented in the Discussion section is frequently overstated. For example, in the first paragraph of this section, the authors suggest this beverage made be used therapeutically to lower cholesterol levels in obese women. However, the degree of cholesterol-lowering observed after consuming this beverage for 4 wk is miniscule. Another example in that same paragraph is “Our study findings add to the scientific basis supporting the cardio protective role of dietary patterns…” This study did not test a dietary pattern, and should in no way imply that it did. - this has been revised.

Furthermore, in the third paragraph, the sentence beginning with “Thus, oxidative stress…can be significantly decreased by dietary intervention with antioxidant-rich freeze-dried fruit powders …” overstates their findings. This study measured only lipid peroxidation in women who consumed a strawberry beverage. – this has been revised.

In the following paragraph, the authors state that “it may be reasonably stated that the improvements in CVD risk factors were due to the strawberry powder…” As there was no control group in this study, the authors cannot accurately state that the effects were due to beverage alone – it is just as likely they were due to chance. - this has been revised.

6. Some of the limitations of this study are stated in the fourth paragraph of the Discussion section. However, the authors neglect to mention the biggest limitation of all – lack of an appropriate control group. – this has been included in the limitations.

7. In the last paragraph of the Discussion section, the authors suggest the need for a future long-term study with a larger population. However, the next step from here is to conduct an RCT, including a well-defined population of adequate sample size. - this has been included in the Discussion section.

8. The study design did not test for any potential mechanisms of action. While other studies that have explored potential mechanisms are mentioned, an extensive discussion of these mechanisms in the context of this study is inappropriate. - this has been revised.

9. With regard to methods, describe why berries, green tea, cocoa, and soy products were the only foods excluded during the intervention. Justify why other foods high in ellagitannins, such as walnuts, were not excluded. - these are the commonly consumed flavonoid –rich foods in our study group. Nuts, like walnuts and
almonds were not the daily/frequently consumed foods and so were not a concern in our study. We did not aim to put the subjects on a polyphenol-restricted diet, but they were instructed to avoid the foods high in flavonoids that they commonly consumed. This has been included in the Methods.

10. Subjects were required to visit their center quite frequently (3 d/wk). Were any interim measures collected? If so, why weren’t they reported?- Subjects were asked to visit the center 3 days/week for the supply of the strawberry drink and they consumed their first cup in the center and took the rest of the supply with them. We did not collect any interim measures other than those at baseline and at 4 weeks of the study.

11. Describe the method for measuring blood pressure in these subjects.- this has been included in the Method section.

12. Was this human intervention trial registered with clinicaltrials.gov or a similar entity? No, this feeding study was not registered with clinicaltrials.gov.

13. How was compliance measured on days when subjects were not monitored? - they were asked to return unconsumed strawberry drink in the containers. This has been included in the Method section.

14. With regard to Table 1, values should be listed per 50g of powder, the dose given to subjects.- this has been revised on Table 1.

15. Why are pre-intervention glucose and triglyceride levels normal in these metabolic syndrome patients? (Table 2)- We followed the criteria of NCEP ATP III in the diagnosis of metabolic syndrome which asks for 3 of 5 features (reference 17) for the assessment of metabolic syndrome. Thus, subjects in our study had 3 features as enlarged waist circumference (> 35 inches), elevated blood pressure (≥ 130/85 mm Hg), and low HDL-cholesterol (<50 mg/dL) as shown in Table 2. Most subjects had normal glucose and triglyceride levels in addition to possessing these 3 features of metabolic syndrome. We excluded subjects on medications for elevated glucose or triglyceride levels.

16. The ellagic acid levels reported in Table 2 are very low. Please state the lower limit of detection for the assay used to assess ellagic acid in this study. It should not be assumed that a non-detectable level is equivalent to 0.- The lower limit of detection in our assay was 3.2 ng/mL.

Minor essential revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1.) In the title, one cannot have pre-intervention effects. Please edit for accuracy.- this has been revised.

2.) In Table 2, values of hsCRP should be expressed as mg/L – this has been corrected.
3.) Please correct reference 18 in the References section. - this has been corrected.

Thank you for the opportunity to improve this manuscript. My coauthors and I will be happy to make additional changes/corrections if needed.

Sincerely,

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