Author's response to reviews

Title: Effect of fruit and vegetable concentrates on endothelial function in metabolic syndrome: A randomized controlled trial

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Version: 2 Date: 13 June 2011

Author's response to reviews: see over
Thank you for the comprehensive review and insightful comments. We have addressed them below, as well as in the text of the document as noted. Our responses are in *italics*.

**Reviewer #1**

I wish the manuscript had line numbers for easy identification.

*Line numbers have been added.*

Major revisions:

Page 5 Para 3: The criteria used for selecting subjects were not exactly based on the guidelines from the AHA/NHLBI. 3 out of 5 should be used for identifying the metsyn phenotype. It is modified criteria. The rationale should be given for deviating from general criteria.

*Thank you for catching our mistake; we have used the International Diabetes Federation (as opposed to the AHA/NHLBI) definition of metabolic syndrome. The text has been revised to note this fact.*

Page 7, para 4: Did the authors test for normality before they used parametric analysis. If the data were not normal, they should use Man Whitney test or Wolcoxin test with Kruskall Wallis.

*We assessed the distribution of our data before selecting the appropriate statistical tools for analysis. Our data were normally distributed. We used parametric statistical tools to analyze our data. We have added the following (line 194): “Data were assessed for normality prior to analysis.”*

Did they measure the dietary intake? It is possible that thier diet may have interferd with the findings. This is a potential limitation and needs to be mentioned along with other limitations. This needs to be discussed. What about the alcohol? Did subjects drink alcohol during the study? This may also have some influence on endothelial function and flow-mediated dialation.

*We did note the limitation of the lack of dietary measures at the end of the discussion section (lines 411-422): “Other limitations include a relatively homogenous sample; largely Caucasian in a suburban community setting, as well as a lack of data regarding dietary, exercise habits, and alcohol intake that could possibly affect endothelial function.”*

I assume that the subjects were asked to maintian thier normal diets (self-selected diets). If so, this needs to be stated.

*We added the following text (lines 205-206): “Subjects continued consuming their usual diet; no dietary advice or guidance was provided during the course of the study.”*

Needs to give a general justification for having a wash out period.

*We added the following text: “As the pharmacokinetics of the tested blends are unknown, our design incorporated an 8-week washout period with no intervention after completing the initial treatment assignment. Subjects then crossed-over to one of the two remaining assignments.” (lines 194-197)*

Needs editing of paragraphs. Some are out of sequence. Needs to arrange in a logical fashion
for enhanced readability and clarity. See below.

*The entire manuscript has been proofread and edited by two external reviewers.*

**Minor points**

Please remove ""precursor" instead use a "risk factor" for type 2 diabetes through the manuscript.

*Done*

Abstract: Please make, "Background and objective" because the "Background" infact has both background and objective.

*Done*

Abstract: Methods, last sentence: add "body" before "weight".

Abstract: Results: Remove the last sentence, "Of note......."

*Done*

Methods: Page 5: Combine #4 and #5 paragraphs with #2. Make one grant paragraph with subheading of "Participants". Completely avoid "one sentence" paragraphs. There were too many 1 or 2 sentence paragraphs.

*Done*

Results: Page 8. Move 1st paragraph to methods. That is methodology not per se results. Start results section with "Baseline characteristics...."

*Done*

Discussion: 1st para, 3rd line. "Endothelial function was fairly normal" I am not sure if that was correct. In the methodology, they described, FMD <10% is considered impaired endothelial function. In the table, the baseline FMD was 8%. That means the subjects were "not fairly normal". They were "fairly on the unhealthy side".

*We removed the text to avoid any ambiguity. We regularly assess subjects (not in this study) who have FMD values of 5% or below that we would consider strongly impaired. Compared to this, FMD in the range of 8% is less impaired.*

Tables: They were very poorly constructed. There are some basics in table building. Atleast draw lines between heading and text and between text and the foot note. Footnotes need to be identified with superscripts in the text of the table.

*We have revised the table structure and footnotes as noted.*

Tables: Please use "Mean plus or minus SD" in 1 column rather than in 2 columns.

*Done*
Tables are stand alone. Table should convey the whole story without referring to the text. Please remove the Table 4. I never seen a one line data table. Please mention this in the text under results.

Done

Figure 1: Please mention actual treatment name instead of "Alterantive treatment assignment".

As this is a crossover trial, the ‘alternative treatment assignment’ can be one of two choices for each arm. With a total of six treatment sequences possible, noting each possible sequence (12P, 1P2, 21P, 2P1, P12, P21) would have made the table difficult to comprehend. We did, however, clarify the ‘alternative treatment assignment’ boxes by noting which treatments were possible (i.e. Blend 1, Blend 2, or Placebo).

Reviewer 2

This study appears to have been well conducted, is of interest, and merits publication. However, the statistical analysis and subsequent interpretation must be revised before resubmission.

Thank you for the kind words; we have revised the document accordingly.

Major Compulsory Revisions

An explanation is required as to why two different blends were used - did the authors expect the effects would be different and why? A total dose per day would be a useful addition.

The two blends were chosen to further assess whether the addition of berries (Blend 1) would result in differential effects compared to a general blend of fruits and vegetables (Blend 2). Lines 218-219 note that “Subjects were instructed to take three capsules twice daily for a total of six capsules per day (4.5 grams).”

Table 1 would benefit from describing the blends more clearly, i.e. just summarising content of the actual interventions rather than original product blends were created from.

Table 1 has been revised accordingly.

2) The main issue with the paper is with the statistical analysis - this alternates between repeated measures ANOVA and comparing changes between intervention groups (correct) and paired samples t-tests, examining within group changes. To use a paired t-test ignores the presence of a control group, and this becomes a single arm intervention study - this therefore must be removed, and interpretation and conclusions based on the correct analysis only. At the moment the conclusion suggests an effect of one of the concentrates on endothelial function, but this is not significant, and does not stand when between group analyses are conducted.

We have removed the discussion on the between-group differences throughout the paper and have only mentioned it once in the ‘ancillary analyses’ section.

Minor Essential Revisions

1) describe different concentrate blends in the Abstract
Done

2) Sentence beginning “Dark berries...” in 2nd paragraph of Background does not fit - rewrite.

Done

3) Fourth paragraph of Background - Aims - first and last sentence repetitive - rewrite.

Done

4) Within methods, inclusion and exclusion criteria should be stated clearly - often repetitive as it stands (e.g. smoking, dietary supplements mentioned as exclusions twice)

Done

5) reproducibility data on the FMD measurement needs to be added.

We have added reproducibility data on our FMD measurements (lines 294-295): “A random sample of 20 FMD measurements were reanalyzed; the correlation (Pearson’s R) between the initial and second assessment was 99%.”

6) from methods it is unclear when the acute effects were tested - this needs to be more clearly stated.

This has been clarified in the Secondary Outcomes section. “Acute (single dose) effects on endothelial function and plasma glucose were assessed by consuming the supplements concurrently with a 75g glucose challenge load and assessing FMD after two hours.”

7) The authors correctly highlight a weakness of the study being a lack of objective markers of compliance - I would like to see this developed i.e. describe the potential impact of this on the study results.

We have added the following to the discussion: “Limitations of this study include the lack of antioxidant biomarkers to corroborate serum antioxidant levels with supplement consumption. It is possible that subjects with low serum antioxidant levels may benefit from supplementation more than persons with normal or supranormal levels.”

8) There are typographical and grammatical errors within the text that should be corrected.

The entire manuscript has been proofread and edited by two external reviewers.

Discretionary Revisions 1) mention fruit and vegetable meta-analyses in intro (Dauchet/He).

This has been cited.