Author’s response to reviews

Title: Dietary Raisin Intake affects Gut Microbiota Composition in Adult Volunteers

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Version: 1 Date: 19 Dec 2018

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RESPONSE to comments made by editor and reviewers

NUTJ-D-18-00241
Dietary Raisin Intake affects Gut Microbiota Composition in Adult Volunteers
Akemi T Wijayabahu; Sheldon G Waugh, PhD; Maria Ukhanova, PhD; Volker Mai, PhD
Nutrition Journal

Dear Dr. Mai,

Your manuscript "Dietary Raisin Intake affects Gut Microbiota Composition in Adult Volunteers" (NUTJ-D-18-00241) has been assessed by our editorial team and peer reviewers. Although it is of interest, we are unable to consider it for publication in its current form. The reviewers have raised a number of points of concern. Addressing these points may allow a revised version to be published in Nutrition Journal.

I also advise that you provide more information on the primary outcome stated in the clinicaltrials.gov submission, and the information your data provides relative to what was stated. In other words, more transparency. As Reviewer 1 pointed out, your results need to be interpreted with caution and without being over-interpreted (as you currently have them).

Response: Thanks for providing us an opportunity to revise. As described in detail below, we have addressed all of the comments made by reviewers. In the revised manuscript we are more transparent and more careful with interpreting our findings. Overall, we feel confident that our
data supports the limited claim that raisin intake can modify microbiota at the OTU level. We are also clearly stating that any potential health benefits remain to be established. Our primary outcome measure was stated as “Changes in diversity of gut microbiota 16S rRNA gene sequences …”. We are using a variety of diversity measures, both alpha and beta diversity measures, in our main analysis. Extrapolations to potential health benefits have been minimized in this revised version.

Reviewer reports:

Reviewer #1: In this manuscript the authors report the findings of an exploratory study looking at the impact of daily raisin consumption for 14 days on the gut microbiome. The primary outcome measure listed on the clinicaltrials.gov website is a "Change in diversity of gut microbiota 16S rRNA gene sequences with regard to time" without a specific mention of what diversity score will be used. It used a pre-post design and recruited 18 participants who were in good health. In the 13 participants who completed the study no changes were seen in any of the diversity measures of the gut microbiome (Chao1, UniFrac, Shannon-Weaver, and Simpson index), which suggests that raisin consumption may not impact gut microbiome diversity overall, however with only 13 participants and no power calculation we do not know what size difference the study was likely to have been able to detect. Phylum level abundance was also not changed by the raisin consumption. Some individual OTUs changed after raisin consumption, however no correction for multiple comparisons were conducted because of the exploratory nature of the study. The manuscript was well written and clear, however there are numerous issues that should be addressed prior to publication. These are listed below with reference to the page and line number.

Response: We appreciate that the reviewer recognizes some limitations in our study design that are due to the exploratory pilot character of our study. We clearly state this limitation as the reason for including p-values unadjusted for multiple analyses. Regardless of the p-value we emphasize that our limited findings need to be repeated in other populations before they can be generalized.

Abstract -in the methods it should say the study was a pilot study or an exploratory study

Response: We modified the “Methods” section of the abstract as follows: “A 14-day exploratory feeding study was conducted with thirteen healthy individuals between the ages of 18 and 59 years.”

Page 3 line 36 to 39. The referenced papers used a raisin extract and not raisins

Response: We revised the sentence to reflect grape products discussed in the cited three articles as follows: “Adding grapes or grape derived products (raisin extracts and grape pomace) to the
diet has been shown to exert potentially beneficial changes in gut microbiota of mice, chicken, lambs and weaning pigs (17-20).

Page 5 line 11/12 Bowel excretion is not a term I had read before, would bowel movement or stool sample be more appropriate?

Response: We replaced “bowel excretion” with “fecal sample” in the following sentence: “The participants provided the first fecal sample on the day before start of raisin consumption (baseline), and after one week (day 7) and two weeks (day 14) of raisin consumption.”

Page 7 line 9, 18 participants were recruited, data from 13 were reported, what happened with the 5 other participants, and explanation and a study flow diagram would be helpful

Response: We have included the participant flow chart in the online supplemental section (Additional File 1: Figure S1) and referred to the figure in the methods section.

Page 8 line 14 to 19. Please remove the statement that says raisin consumption increased the relative abundance of Bacteroidetes etc, you cannot say there was a change if the change did not meet the threshold of significance you have set in your methods.

Response: We agree and modified this statement to: “While there were indications for a trend towards an increased relative abundance of Bacteroidetes and decreased relative abundance of Firmicutes, these observations at the phylum level did not reach statistical significance.”

Page 9 line 45-48 please support the line regarding reduced risk of subclinical enteric inflammation and UTI with references.

Response: The increased presence of an opportunistic pathogen, Klebsiella in this case, suggests to us increased risk for infection in targeted anatomic sites. We provide now references to provide appropriate background.

[33] Lin et al, provides evidence for the presence of K1, K2, K20, K54, non-typable serotypes of Klebsiella pneumoniae isolated from fecal samples of healthy adults (N=76) and the presence of these serotypes in urinary isolates from UTI patients (N=54) and pneumonia patients (N=29).

[34] Ebrahimi et al, provide evidence for asymptomatic fecal carriage of extended spectrum beta lactamase producing Enterobacteriaceae in healthy individuals which includes Klebsiella pneumoniae.

[35] A review article written by Davis and Lance discussess potential sources of Klebsiella pneumoniae enteric colonization among healthy adults.
Page 10 line 38 to 43, I am unconvinced that the OTU level changes may only be a conservative estimate of the impact of raisins. While the limited number of participants and the factors which contribute to your variability make it harder to detect an effect, it also increases the risk of seeing a difference based on chance, especially given there was no corrections for the multiple comparisons conducted.

Response: We agree and removed the sentence.

Page 10 line 43 to 49. This sentence seems contradictory to me. You say your specific findings (I am assuming the OTU changes) cannot be extrapolated to other populations, but that raisin consumption likely changes microbial composition is generalizable, but none of the composition diversity or phylum level measures were changed in this study.

Response: We clarified as follows: “While the specific OTU’s that changed upon raisin intake in our small study might not be observed in other populations we suspect that some OTU level changes would occur in other populations.”

Page 10 line 49 to 54. I think the suggestion that the data from this study would indicate potential health benefits of raisin consumption is premature and goes beyond what the data support.

Conclusion. Similar to the comment above I am not convinced the results of the study can modify the fecal microbiota towards a more beneficial composition. This is due in part because the changes in the study were very minor if any, and the link between individuals OUT changes and health is also of lower quality.

Response: We agree and have accordingly modified the concluding paragraph and the last sentence “However, it remains to be shown in future studies that include well-defined health outcomes if any microbiota changes resulting from raisin intake correlate with health benefits.”

Reviewer #2: Wijayabahu et al. report on the changes in the microbiota composition of healthy volunteers after consumption of raisins. The topic is interesting and the research well conducted. It would have been interesting to look at other physical/chemical parameters in the collected faecal samples, including short chain fatty acid production. It is not clear whether consumption of raisins can be considered beneficial, the authors report a decrease in Bifidobacterim sp. A statement including a conclusive aspect could be useful.

Response: Please see above, we have addressed this concern. Regarding Bifidobacteria we have clarified by adding: “We did not detect a consistent increase in Bifidobacteria or Lactic Acid Bacteria, often considered beneficial gut microbes. This could partially be due to amplification
bias as Kuczynski et al reported that 16S rDNA Illumina primers are less efficient in amplifying Bifidobacterium spp. (32).”