Author's response to reviews

Title: Associations between dietary habits and body mass index with gut microbiota composition and fecal water genotoxicity: an observational study in African American and Caucasian American volunteers

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

Please find enclosed our revised manuscript MS: 1555553326283883, entitled “DIET, GUT MICROBIOTA COMPOSITION AND FECAL GENOTOXICITY IN AFRICAN AND CAUCASIAN AMERICAN RESIDENTS OF THE EASTERN SHORE OF MARYLAND” for publication in Nutrition Journal. Neither the submitted paper, nor any similar paper, other than an abstract or preliminary communication, has been or will be submitted to any other primary scientific journal. All the authors are aware of and agree to the content of the paper and to being listed as an author on the paper, and there are no financial or other interests to disclose.

We have carefully revised the manuscript according to the suggestions by the reviewers. We feel strongly that these revisions have helped to improve clarity of the manuscript. A point by point response is below.

Please let me know if there are any remaining concerns.

Best regards,

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Reviewer # 1

Reviewer comments:

a) There is no mention of DGGE profiling in the results nor in the discussion. Authors need to include data or remove the method if there is no need for data.

Response: The first paragraph under “Microbiota analysis” describes the results of the DGGE analysis, we added: “Based on DGGE profiles, the mean Shannon-Wiener diversity …” to make this even more clear

b) There is mention of qPCR in the method section but authors do not present
results i.e. data not shown. Authors should present the data in a comparative Table or Figure with the FISH analyses and correlated with faecal water analyses to strengthen the data showing no changes in have no results presented on either approach in the paper. This is an attention to detail that the authors should have checked prior to submission.

Response: We added the qPCR data in Figure 3b, allowing for a comparison between the two methods that principally yield the same result.

Minor essential Revisions

page 6: Study design
line 3 Target population: authors need to mention BMI for division of subjects into lean and obese. Done

line 8: "within the past 4 weeks" Done

line 9: rather than "We enrolled..." state "A total of 98 subjects, 52 AA and 46 CA were enrolled" Also is it 98 or 101 enrolled? Done

line 15 states a 4 day record. How valid is this compared with 7 day FFQ? We used two methods to validate the FFQ, neither method is perfect but our observation that results using either method yields consistent results is reassuring.

line 23 Analytical cohort states 101 subjects so is it 98 or 101? 98, changed

page 7
line 2. Authors need to mention BMI measurements of the AA and CA groups and subgroups. Done

line 13 Denaturing Gradient Gel Electrophoresis: delete or put results in it adds to paper. DGGE discussed, see above

line 24 Fluorescent not Fluorescent. Done

page 8
Authors need to justify why they used DAPI for total bacteria rather than Bact 338 Fish probe. There is quite a bit of difference in total bacteria number using either probe. Authors to comment. We added: “DAPI was used as it allows for the enumeration of total bacteria without the need for a separate hybridization.”

line 18: clarify by stating "two bacterial groups" Done

line 20 qPCR: Authors should explain where the subset of lean and obese
subjects come from. This is not made clear. Made clear now in methods section: All 14 lean study subjects (BMI <25) and 14 randomly chosen obese subjects (BMI >30) were included in the studies of the association between microbiota and obesity.

page 9
line 20: Human tumor cell line HT29.
Was this better than Caco-2 cells for toxicity assays? Authors should explain. HT29 has been used previously by the lab. Results might differ for any cell line and there are many comparable cell lines that would be available.

line 23 "thawed" not "thawn" Done

page 10
line 1: Why the difference in passage numbers for cytotoxicity versus challenge assay. Challenge data not clear on page 12. Challenge assay was performed on the same cells AFTER cytotoxicity was determined. We clarified as follows: “… we analyzed the effects of preincubation of HT29 cells with fecal water before challenge with H2O2 and measurement of its genotoxicity

Line 21 "Multivariate regression analysis (SAS0 was used" rather than "We used multivariate..." Done

line 24 What are significance values set at p<0.05 or p< 0.1? Authors should state this. Done, added (p <0.05).

page 11
Dietary analysis
line 10: Authors should state significance value for results e.g. MeIQx is at p<0.05. Done

Feacal water analysis
line 16 "FW was performed in a subset of 21AA and 22CA subjects...". How is the subset determined i.e. BMI of lean versus obese? Authors need to state. This was limited by the amount of fecal water we obtained. We do state: “for which we had a sufficient amount of fecal sample"

page 12
line 2"between the two racial groups....". Is this the subsets then say so. No as we state, these are the AA and CA racial groups.

line 6: "two groups" should be "two racial groups" Done

line 7: "Furthermore detected a..." should be "Furthermore, the results showed a...” Done

line 11: "limited number of subjects”. How many? Authors should state number.
We do state that the analysis was done in 21 AA and 22 CA, which we consider a limited number.

line 20 Authors need to include "Shannon-Wienmer diversity index" in page 10 Stats section of Methods. Done

page 13
line 5. What is Bacteroidetes level compared with Clostridia?. What is ratio of Bacteroidetes: Firmicutes? Does this differ to references 49 and 50? Would this data have been better with a Bact 338 probe for total bacteria.
We now show that FISH and qPCR (New Fig. 3b) both show no difference between lean and obese, supporting the argument that using the Bact338 probe to determine “all bacteria’ would unlikely affect our results.

line 17 real time PCr (data not shown). this data should be shown as it strengthens authors argument that microbiota profile in lean and obese subjects does not conform to Ley et al (50) analysis of smaller human population. This is very important data. and should be included in a Table or Figure (e.g. figure 4) comparing FISH analyses with qPCR to show similarity in data.
We agree, and have added Fig.3b to show the qPCR data

Discussion
Page 15: No results for PCR-DGGE mentioned in results or discussion. Authors either put the data in or leave out the method.
We are presenting the DGGE data and the diversity index derived from it in the first paragraph of “Microbiota analysis”

Table 1 page 16. Authors should put data in on Subgroups of lean versus obese so this aligns with FISH analyses
Table 1 describes the overall study population as these characteristics are necessary to evaluate the associations under investigation. The obesity substudy is limited to studies of associations between obesity and microbiota composition, the data presented in table 1 have little relevance and are not discussed in that analysis.

Table 2 page 17 Authors should put in significance values at bottom of table.
Moving the p-values to the bottom rather than leaving them in a separate column would make it more difficult for the viewer to see the significance of differences between the two groups fro each of the presented values

page 23 Figure legends

Line 2 Figure 1, correct position of information Done

Figure 2 Was the Bact338 probe used as alternative to DAPI as this may change data. No, we prefer to use DAPI to decrease bias introduced by hybridization
Reviewer # 2

All concerns raised by reviewer # 2 have been addressed and incorporated into the revised manuscript as described above.

Reviewer # 3

There are some suggestions/comments about this manuscript:

1) Description of study design was entirely unclear. The study set should be more carefully described to make the reader understand. This should be clarified by a new figure on the study set up and sampling times.

We agree, and have added a separate heading (Study logistics) in the Materials and Methods section:

“Study logistics

Subjects were approached about the study through local community groups. After enrollment, subjects received a study kit that included all questionnaires as well as a stool collection kit. After filling out the questionnaires subjects kept a four day food record and collected the first stool sample after its completion.”

2) The authors should be clarifying the FISH’s data as Log10 Cell/g of stool instead of Bacteria/g of stool.

Log transformation of the raw FISH data would suggest that there is no association between diet, race and microbiota composition, mainly due to the large variation that would require a huge sample size to establish significance. We clearly acknowledge this in the manuscript. The raw counts allow us to present preliminary evidence that microbiota might be associated with diet as well as race. It is currently unclear how diet affects microbiota, our data provides early evidence for the existence of some associations.