Author's response to reviews

Title: Effect of dietary prebiotic supplementation on advanced glycation, insulin resistance and inflammatory biomarkers in adults with pre-diabetes: a study protocol for a double-blind placebo-controlled randomised crossover clinical trial

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Author's response to reviews: see over
Response to Reviewers

23 June, 2014

Dear Editorial Staff


Thank you for your positive response to this article. We would like to thank the reviewers for their constructive comments, which have been addressed in the table below. Changes to the manuscript have been highlighted in red font.

We have carefully considered the comments provided by the reviewers, and have provided our response below:

<table>
<thead>
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<th>Comments from Reviewer 2</th>
<th>Response from Authors</th>
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| **Major compulsory revisions:**  
1. Is a two-week wash-out period long enough to be sure that microbiota return to its initial profile, without any resilience, for the group beginning by prebiotic intervention? It should be useful to plan blood, urine, and particularly stool analyses at the beginning of the second period of intervention. | Previous studies have demonstrated that dietary modification induces changes to gut microbial growth within a relatively short time frame (14-15 days) (Gibson 1995, Kolida 2007)  
In order to minimise participant burden, we have limited the collection of blood, urine and stool samples to three occasions during this trial. We will use statistical analyses to test for carry-over effects. |
| 2. The sample size seems to be low. It is calculated based only on the objective to detect a reduction of CML concentration (p7) although the announced objective is to investigate the effect of prebiotic on gut microbiota and AGE accumulation (p6). Moreover, in the abstract methylglyoxal levels differences between treatments is noted as primary outcomes, but it is not included in the sample size calculation. If regarding CML levels and reduction objective, the sample size calculation appears correct; regarding gut microbiota, this sample size appears too low to take into account the diversity of the gut microbiota. | The sample size required for this study was calculated based on the primary outcome (change in CML and methylglyoxal concentrations). This outcome is what makes this study unique as it has not been investigated previously. Changes in gut microbial growth is a secondary outcome which will provide preliminary data which may provide a basis for future studies. Therefore, changes to gut microbiota have not been included in sample size calculations. |
3. The authors plan to analyze only bifidobacteria and lactobacilli populations although publications involved also other bacterial groups in metabolic disorders (recently reviewed by Delzenne et al, Br J Nutr, 2013,109:S81-S85). To assess the announced aim, the authors should include other PCR to detect the major bacterial groups, including those suggested to be involved in metabolic disorders and those suggested by the authors to be involved in AGE metabolism (p5).

Investigation into changes in colonic bacterial growth following prebiotic supplementation is a secondary outcome of this study, because time and funding constraints prevent a full microbial analysis of stool samples belonging to large numbers of research participants. Instead, we feel it is more economical to explore the growth of a limited number of colonic microbial species, the results of which may suggest avenues for future study.

However, based on the reviewer’s suggestion, we have now included qPCR analyses for some additional bacterial species suggested to play a role in the modification of host metabolism including *Roseburia* spp., *Faecalbacterium prausnitzii* and *Akkermansia muciniphila*.

4. To validate their choice concerning the primers used, they must give the references of the primers used.

References for the PCR primers have now been included on page 12.

5. The aim of the study should be therefore reconsidered. To my point of view, it is very interesting to complete biochemical dosages with appropriate microbiota determination.

We have reworded the aim of this study on page 6 to better reflect its primary objective (investigation of changes in advanced glycation) and one of its secondary objectives (exploration of changes in the growth of a limited number of gut microbial species):

“This trial was designed to investigate the effect of a prebiotic dietary supplement on AGE accumulation and explore changes to the growth and activity of specific gut microbiota in adults diagnosed with prediabetes”.

6. As far as exclusion criteria are concerned, how long before the inclusion visit the patients should they had not taken antibiotics of pre/probiotics supplements? Otherwise, will guidelines for eating be given taking into account that numerous foodstuffs contain pre- and/or probiotics?

We have amended the exclusion criteria on page 7 to exclude: “individuals who have taken antibiotics, dietary prebiotic or probiotic nutritional supplements within the previous three months”.

As we are interested in changes in AGE levels from baseline, we will not be providing participants with specific dietary guidelines, other than to “otherwise maintain their usual dietary intake and level of physical activity” (page 9).

Minor essential revisions:
7. Could the authors explain why the

We have now included the following explanation for the gradual increase in
prebiotic intake should be given with a gradually increased dose over ten days until the target dose is reached? Is it usual in prebiotic supplementation clinical trials (to my knowledge…no, but I did not check this point). | supplement/placebo intake on page 8 of the manuscript: This stepped escalation in supplement dose aims to minimise gastrointestinal discomfort for participants, as a sudden increase in dietary prebiotic intake may result in increased stool frequency, abdominal bloating and flatulence until the bowel adapts to the increased fibre intake (Causey, 2000). Other clinical trials where prebiotic intake has been gradually increased over 1-2 weeks in order to minimise gastrointestinal side effects include Parnell (2009), Dewulf (2012) and Pederson (2013).

| 8.Currently, it is better to use the term "microbiota" instead of "microflora". | The term microflora has been replaced by the term microbiota and is highlighted in red font in the manuscript. |

Thank you for inviting us to resubmit this article and we look forward to hearing from you in the near future.

Yours sincerely

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References:


