Dear Navjoyt,
Please find below a point by point reply to the editors’ and reviewers’ comments. We thank the reviewers and editors for their constructive suggestions and think that the manuscript has been considerably improved as a result, and hope that it will be now suitable for publication at the BMJ.
On behalf of all authors,

Ana Valdes

Editors’ comments.
1) Many thanks for your hard work on the paper. Editors felt that it gave a very good overview of what we know and what we don’t know about the gut microbiome and health. The comments here are intended to make the evidence base and level of current understanding clearer for a general medical readership, as well as strengthening the background, definitions, and take-home messages for readers.

2) We wondered if a discussion of the quality of evidence should be included early on the article. Animal studies are frequently cited, as are studies of surrogate markers, and we thought it would be helpful to give general readers some steer about how certain we can be about current understanding. We felt that a subsection on this within the main text towards the beginning would help orientate readers as they go on to read about the evidence, but you may wish to include a box on this instead.

Authors’ response. We have added a paragraph in the introduction in this regard (the fourth paragraph of the text).

3) We noted that there were several instances where qualitative descriptors were used to describe outcomes of studies (for example, “Protective, positive, useful, “change the function of”, “act directly on the host”). We think it would be helpful to be clearer when reporting the outcomes of studies. For example here: “IPA is a microbiota-produced product highly correlated with dietary fibre intake ... that exerts neuroprotective effects against Alzheimers 23...” BMJ Readers are likely to take away a different understanding of neuroprotective effects against Alzheimers compared to what is described in ref 23 which was an in vitro assay using primary rat cells and a neuroblastoma cell line.

Authors’ response. We have modified these sections in the text using more accurate descriptors.

4) On related note, microbiome composition and bacteria population counts are common outcomes in the studies cited, and it would be useful to provide more comment on what this tells us about health. Is there evidence to support the associations between greater microbiome diversity/populations on clinical outcomes that can be cited? Are there any limitations that might help a general reader interpret microbiome composition as a surrogate marker for health?

Authors’ response. We have added references regarding the various disease conditions associated with microbiota diversity and added some limitations. Moreover we have added a sentence on the functional role of diversity as shown in RCTs of FMT

5) The table on what we know and don’t know is very helpful, but it was sometimes hard to find supporting evidence for “what we know” within the text or any comment on the strength/quality of
evidence - can this be made clearer or added where relevant (either within the text or the table could be referenced)?

Authors’ response. We have added the relevant references to this table

6) One of the key aims is for readers to be able to take away practical information to inform practice and policy. To this end, we wondered if a table outlining key food/nutrients and the evidence for benefits would be helpful (Fibre, Cheese, FODMAPs for example)

Authors’ response. We have added a table with some examples of this information.

7) We agreed with one of the reviewers’ about fibre perhaps being a key message. You say in their conclusion: “Fibre is a key nutrient for a healthy microbiome, and has been side-lined in recent debates on sugar and fat. There has been a lack of investment in clinical trials of natural or synthetic fibres, despite the fact that the low-fibre western diet has been linked to the depletion of the industrialized microbiome” - this does seem important and could be more prominent?

Authors’ response. We have stressed the importance of fibre within the key messages as per the reviewer’s suggestion.

8) You mention directions for future research and current uncertainties throughout the text, but perhaps this could be more clearly wrapped up at the end to give a stronger conclusion and way forward. Given any current gaps in evidence and practice, how we can achieve more definitive answers in the future?

Authors’ response. We have added a final sentence: “Given the current gaps in our knowledge (evidence), there is an urgent need for clinical evidence that can be translated into clinical practice. To achieve this, randomised controlled studies using consistent matrices of prebiotics or probiotics or FMT are needed to assess changes in gut microbiota composition and in health outcomes. “ Streamlining of bureaucratic obstacles to these non-pharma treatments may be needed to overcome this

9) We wondered if some additional background might be helpful for the general reader. What influences the make up of the microbiome - what proportion is genetic and how much to do with the food we eat? What are some of the key components of the gut microbiome? A box with a glossary might also be helpful for terms such as microbiome, microbiota, colonocytes, plus any others that might not be well-known to general readers.

Authors’ response. Authors response, we have added a paragraph (second paragraph) on heritability and the contribution of genes compared to diet and environment but gut microbiota composition. We have also added a glossary with this and other terms that may not be well-known to general readers.

We hope that you will be willing to revise your manuscript and submit it within 4 weeks (i.e. by Monday 16 April 2018).

When submitting your revised manuscript please provide a point by point response to our comments and those of any reviewers. We also ask that you keep the revised manuscript within the
Authors' response. Given the considerable amount of additional text that the editors have requested us to add we have done our best to stay under 3250 words by cutting other sections.

Reviewer(s)' Comments to Author:

Reviewer: 1

Comments:
Dear authors,
Dear Dr. Ladher,

Thanks for contacting me.
I read with interest this review. It is a well-written, well-balanced, up-to-date yet concise review on the topic. The authors used human studies and perform research for systematic reviews in the field. Table 2 is very informative and limitations of these systematic reviews are well discussed in the text.
I have a few minor suggestions and comments.
Authors' response: we thank Dr Bindels for her positive assessment of our manuscript.

- On several occasions, I would replace the term “microbiome” by “microbiota”, including in the title and in key message 1. As the authors explained it at the beginning of the text, the microbiome encompass the microbial genes, whereas the microbiota refers to the community, which for me is essential. What matters is more to change in a beneficial way the functionality of the microbiota, the panel of metabolites produced than the composition of the genes. Although the second often leads to the first, it is not a preliminary.
Authors' response: we have replaced microbiome for microbiota except where it refers to the genome of organisms

- One of the key messages and conclusions of the manuscript is that “the medical community needs to adapt their education and public health messages accordingly”.
What would be the public health message that could be conveyed right now, based on our current knowledge of the gut microbiota?
Authors' response: We have added this to the key messages

I completely agree with the authors that currently, we don’t have enough good quality information to provide recommendations on type, dose, and duration for probiotics. What we can say for sure is that fibre consumption is associated with beneficial effects in several contexts. Should this be the message to reinforce?
Authors' response: Authors’ response: we agree with the reviewer and have added this as suggested to the key messages

- Page 9: the transition from fiber to probiotic food is quite abrupt, maybe subsections may help the reader to follow the flow. As to my understanding the meta-analyses include probiotics with different matrices, I am not sure how important the concept of probiotic food is here.
Authors' response: We have made the subsections more clear

- BOX2, “prebiotics better referred to as MAC”. Although there has been quite a debate in the
field on what is a prebiotic and what is not, none of the proposed definitions restrain prebiotics to carbohydrates. I think MAC and prebiotics are overlapping not identical concepts.

**Authors’ response:** we have removed the text “better referred to as MAC” and addressed the fact that the definitions are limited and are a topic of current debate

- The title of the first section “Role of the gut microbiome in nutrition and obesity” does not reflect the content of the paragraph. This section is all about metabolic products of the gut microbiota.

**Authors’ response:** we have removed the subtitle

- Page 3: most studies of humans show a dysbiosis characterized by a lower diversity in overweight and obese subjects. It might be good to provide one of two references.

**Authors’ response:** We have provided a number of references on dysbiosis and loss of diversity for various disease states

- Stating that IPA exerts neuroprotective effect in Alzheimer disease and cerebral ischemia based on ref 23 (page 3) is a bit of shortcut. Ref 23 presents in vitro data on the scavenging properties of IPA.

**Authors’ response:** This has been rephrased

- Page 4: ref 26 on DSS might not be the most appropriate one to refer to the mechanisms by which gut microbiota dysbiosis promotes WD-induced obesity.

**Authors’ response:** we have replaced this reference with Boathman et al Lipids Health Dis 2016 and Gabele et al 2011 (refs 29 and 30)

- Page 6: ref 48 does not describe MAC as stated in lines ~43-47.

**Authors’ response:** we have replaced this reference with Sonnenbur cell Metabolism 2011, Deehan et al Microbiol Spectr 2017, Bindels et al Nat Rev GastrEnter 205 and Walter Cell Host Microbe 2015

- Page 11: the subsentence “with ever larger populations acquiring microbiome data” is not clear to me. – this has been corrected

I found out of few typos:

- TMA is trimethylamine, not trimethylamine N-oxide (page 3) – this has been changed
- “Alzheimer disease” instead of “Alzheimers” (page 3) - deleted
- Last sentence on page 3 would benefit from rephrasing. – this has been rewritten
- Several points are missing,
- Page 5, Verrucomicrobia phylum correct
- Page 5, “on the presence of gut microbes” – correct
- Key messages : “the gut microbiome composition and its manipulation influence”- correct
- Key messages : “the medical community needs”- correct
- Table 1, point 2: “the microbes in our gut influence human energy metabolism - correct

I sincerely hope you will find my comments helpful,
Do not hesitate to contact me if needed,

Best wishes,

Laure Bindels

Reviewer: 2
Recommendation:

Comments:
The Analysis article titled “The role of the gut microbiome in nutrition and health” discusses the highly relevant and interesting role that diet plays on the microbiota composition and function and how they impact human health. This topic is of interest to both specialists and the general scientific community. The article synthesizes much of the recent literature of both animal and human dietary studies that have demonstrated links between diet, food additives, medication, and supplements and the microbiota with a number of diseases including obesity, cardiovascular disease, type 2 diabetes, etc. The authors do a nice job delineating the complexity and challenges of dietary intervention studies in humans, as well as provide a synopsis of many of the most relevant studies done to date. They also point to encouraging preliminary work that lays the foundation for future study of human subjects. The authors also summarized trials of probiotic supplementation to produce a couple helpful tables of demonstrated benefits and outstanding questions related to probiotics as well as a summary the key results for the studies.

Authors’ response: we thank Dr Sonnenburg for the positive comments on our manuscript.

One notable study that was not discussed in the article is the 2016 Wu et al study comparing the microbiota and metabolome of vegans and omnivores. This study would fit well in the section discussing popular diets and nicely demonstrates how the lack of reproducible microbiota differences between individuals on different diets may mask differences that can be seen in the metabolites produced by the microbiota.

Authors’ response: we have now added the following paragraph to the “popular diets” section as suggested by Dr Sonnenburg .

In the study from Wu et al 2016 [PMID: 25431456] a cross-sectional comparison of 15 vegans vs 16 omnivores found striking differences in the levels of serum metabolites generated by the gut microbes, but very modest differences in gut bacterial community composition. A controlled feeding experiment of 10 omnivores, half of them randomised to a high fat/low fibre diet and the other half to low fat/high fibre for 10 days found very modest effects on gut microbiome composition and no difference in SCFA production. These data support a greater role for diet as a substrate influencing short term the bacterial-derived metabolome rather than the bacterial composition, which could be more important functionally.

In Figure 1 there are various dietary inputs used to contrast health and disease outcomes. TMAO production is one of the deleterious outcomes yet excessive protein consumption is not listed as an “input”. This figure is also somewhat misleading in that it appears that all deleterious outcomes are a result of TMAO production. Authors should consider adding other microbiome outputs to this figure (ie reduced SCFA production, other microbiota produced metabolites as described in studies by the Fischbach group)

Authors’ response: We agree that, although our intention was simply to include examples of mechanisms whereby the gut microbiome influences human health, it needs further detail. We have therefore modified Figure 1 to include other diet inputs and other microbiome outputs such as LPS and indoxil sulfate which have been investigated by the Fischbach group

One minor point is the use of microbiota vs microbiome. The authors nicely define microbiome as the collective genome of microorganisms, however in the manuscript the authors will use microbiome when referring to the collection of organisms, not their genomes (for example lines 3-7 on page 4; Box 1 that defines microbiome diversity as the measure of how many species ...; microbiome vs microbiota accessible carbohydrates in manuscript vs Box 2). The field is already
mired in the confusing use these terms, this might be a good place to try to delineate some clearer definitions.

Authors’ response: We have modified the text to consistently use microbiota when referring to the organisms.

Additional Questions:
Please enter your name: Erica Sonnenburg

Job Title: Senior Research Scientist

Institution: Stanford University