Reviewer’s report

Title: Associations between dietary micronutrient intake and molecular-Bacterial Vaginosis

Version: 0 Date: 15 Aug 2019

Reviewer: Gerald Murray

Reviewer's report:

Tuddenham et al. present a study examining the association between micronutrient intake and molecular bacterial vaginosis, finding that betaine intake was associated with BV. It is important that studies that were not optimally designed with microbiota analysis in mind (i.e. a retrofit of microbiota analysis to stored samples from a clinical trial or observational study) can still be used so long as they present novel findings and are couched in appropriate caveats (e.g. noting cross-sectional design, small sample size). I feel that the authors have achieved this.

For those unfamiliar with the food questionnaire, it would be beneficial to provide more information on this. For example, how reliable are intake estimates from this methodology, and what period of time is captured in the result (noting that dietary changes can have a rapid impact on gut microbiota, and vaginal microbiota can rapidly change) - these may enter the discussion as a potential limitation.

Is there a standard list of micronutrients that are assessed in the questionnaire? How many micronutrients were considered in this study? Table 2 lists 16 nutrients but there are probably more (e.g. iodine, B2, copper) which are not listed - were these also examined?

For methods, vaginal microbiota characterisation. How many samples underwent a second round of extraction/sequencing, and could this have affected results? Is there validation data indicating that the latter extraction method gives improved results, noting that it has a lower input volume? Is the suggestion that these samples contained PCR inhibitors, or they had low DNA content?

Can the authors please indicate the year of recruitment of participants and the duration of storage of samples prior to extraction and analysis. Is there a reference for the parent study?

There could be some more detail on the sequence results (e.g. what was the range and average read number for samples).

For negative controls, presumably there were no swabs from the original study to use as negatives? The methods should detail how the negative controls were used to inform the analysis of study samples - i.e., what was present in these samples, and what was subtracted from the results of study samples. This information will help others when performing similar analysis.
Other factors associated with BV were not included in the analysis (e.g. past history of BV, female sexual partner, partner condom use), and factors that may have been important (e.g. recent antibiotic use, menses). Data may not have been collected for these factors, nevertheless these data should be mentioned as unavailable.

Were samples used in this study also assessed for BV by another method, and if so, how did that correlate with the "molecular BV" designation?

For the designation for each community state type, can the authors indicate the proportion of the main taxon in each group?

In the discussion, it would be helpful to include some more information on some of the studies - for example, samples sizes and strength of associations could be indicated in parentheses "(n=X, aOR=Y, p=Z)" to help the reader assess the outcomes of the study without downloading the supplementary table.

Could the authors expand their discussion of betaine slightly? E.g. is it fully digested/absorbed by the gut, or does a substantial amount pass through? Is it secreted/available for bacteria at skin and mucosal surfaces?

The authors note that betaine may enhance the osmotollerance of Lactobacillus species, but could this be a common phenomenon, i.e. there evidence of betaine supporting other species, and could this extend to G. vaginalis and other BV-associated bacteria?

Minor comments:

It would be good to list the number of CST (line 8, paragraph 2 of background)

A few minor errors in Table 1: 49/78=62.8%, 12/26=46.2%

"FFQ" should be defined (used in Discussion)

In the discussion about the study by Neggers et al., the direction of the associations with folate, Vit E and Ca could be indicated.

This is a well written and easy to read manuscript

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