Reviewer’s report

Title: PCOS without hyperandrogenism is Associated with Higher Plasma Trimethylamine N Oxide Levels

Version: 0 Date: 28 Oct 2019

Reviewer: Flavio Cadegiani

Reviewer's report:

Authors did a great job as they significantly improved the level of the manuscript, in particular the discussion, putting their findings into a great context. Congratulations for the improvements (and for the study itself).

Some points that I would like to bring, not as points to be corrected, but to be added, if the authors consider that they make sense, in the discussion:

1. Since there are no reports of TMAO levels in hyperandrogenic PCOS women, why didn't the authors perform a third group of hyperandrogenic PCOS women?
2. TNF-alpha, besides only increase with weight when in PCOS, is an independent marker of PCOS, according to this study. Could this be also highlighted?
3. Overweight women has a biochemical phenotype that resembles the PCOS group for some parameters, although TMAO remains a specific marker for PCOS, even among those overweight.
4. IL-10 and IL-33 seem to be reduced in PCOS-NA when overweight, compared to the other three sub-groups. Would these markers indicate exacerbation of the additional risk for women with PCOS when they gain weight, compared to the risk of weight gain among women without PCOS? Since we know that besides the inherent increased risk of obesity when under PCOS, the risk of being overweight is even higher when under PCOS, when compared to the risk of being overweight in non-PCOS.
5. Also, once CRP increased with weight only among PCOS women, but not among women not affected by PCOS, is this an additional demonstration why the weight gain brings even more risk when the weight gain occurs in PCOS women, compared to the risk of gaining weight of non-PCOS women?
6. The fact that women with PCOS should be even more careful to prevent weight gain, due to the risks that are exacerbated in this group, should be highlighted in this paper, since it shown novel markers of the additional risks from the "combination PCOS+overweight", compared to PCOS or overweight alone. This indicated that policies for prevention of obesity should be even stronger in the group of PCOS.
7. Although some markers including HOMA-IR were shown to be higher in PCOS, compared to non-PCOS, TMAO and LH/FSH are the two most specific markers for PCOS found in the study, which is reinforced by the fact that even normal weight PCOS had higher levels compared to overweight non-PCOS subgroup. These markers, that show that even non-hyperandrogenic women with PCOS have particular features that are not prevent in non-PCOS, even when overweight, seem to be the first ones
to be shown to be true and accurate markers for PCOS, which has particular importance among women with PCOS without hyperandrogenism, since this particular group is challenging to be diagnosed, due to the lack of hyperandrogenism.

8. Does IL-17A play an important role in the levels of TMAO when overweight only when non-PCOS? Does it mean that IL-17A "relatively loses" its importance for the levels of TMAO when under PCOS? This does not mean that IL-17A does not have a role in the PCOS, but would have a weaker correlation with TMAO when under PCOS, instead.

9. The last paragraph of the discussion seems to be an introduction of what TMAO is, which should be placed before the "final discussion and limitations" (which is located in the paragraph immediately before this one). My suggestion is that authors could either rewrite the paragraph for a logical sequence: "Since TMAO has multiple effects, including human thrombosis and platelet formation, inflammation, lipid metabolism, IR, glucose metabolism, the development of cancer as well as the occurrence and development of related diseases, and has been studied in cardiovascular disease, it is expected to be a predictor for early diagnosis, efficient evaluation and prognosis of the above diseases, and may become a therapeutic target for related diseases. In this context, is TMAO associated with the pathogenesis of PCOS, or could its levels be a consequence of PCOS, in a similar manner to TMAO being a consequence of increased weight? Further research on the role of TMAO may provide new ideas for the diagnosis, disease monitoring and clinical treatment of related diseases." OR, authors could replace this paragraph, OR divide into two, leaving the intriguing and great questions in the end after the final discussion, and the first part before the paragraph before (always adjusting to maintain the logical sense).

In addition, there are some specific points to be addressed, as below:

Abstract:
- Results: since "FINS" is less known than the IL and IFN, please specify what this abbreviation means.

Page 5 Lines 13-15: Objectives: "whether plasma TMAO levels ARE correlated with PCOS without HA" - th

Page 5 Lines 29-30: "Existing studies have demonstrated that BMI is associated with increased concentrations of TMAO in plasma" -

The materials and methods sections should be left for the objective explanation on the subject selection criteria, procedures, etc. The justifications for specific choices of any of the characteristics of the materials and methods should be either in the discussion: "We divided into 4 subgroups because TMAO has been associated with BMI...", or in the introduction, since if this statement ("BMI is associated with TMAO levels") is present in the introduction, the choice for the division proposed in the study is already justified and does not require further explanations.

Page 6 Lines 1-2: "There was no significant difference in age between the groups (P > 0.05)." - This should be replaced to the baseline characteristics of the results section.
Page 6 Line 7-16: It is important to specify the assay for each of the baseline blood markers, particularly in the case of: 1. Testosterone, once this was used as a criteria for HA/non-HA, and testosterone for females dosed by immunoassays lacks precision, which may compromise the selection of the subjects, and 2. Small Dense LDL (sdLDL), for which the assay had been required in the review of the first version of the manuscript, but authors did not mention in the second version. Please specify the assays employed for all parameters.

Page 7 Line 14: Please change the subheading from "Increased plasma levels of TMAO and inflammatory factors in the PCOS-N group" to "Increased plasma levels of TMAO and inflammatory factors". The results should not be made explicit within the subheading, but in the text that follows the subheading instead.

Page 7 Line 32: "That is, a higher plasma TMAO level or LH/FSH ratio increases the risk of PCOS." Authors did a great work on performing the adjustments for BMI and the logistic regression. However, in regards with this specific sentence, I have two points: a. This seems to be a conclusion of the findings of the results. Hence, this would be better placed in the discussion or conclusion. Results should be limited to the objective findings, not analysis. b. Do the authors mean that higher TMAO levels or LH/FSH ratio increases the risk of PCOS, or that these parameters independently indicate the presence of PCOS? As far as I could interpret, increased TMAO levels and LH/FSH ratio 3.8 and 18.0 times more present in PCOS, compared to non-PCOS women. However, I was able to understand the sentence above when I read the discussion section. Thus, I suggest the following sentence for the results sections (which is a strict and objective interpretation of the results): "Females with higher plasma TMAO level and LH/FSH ratio are 3.8 and 18.0 times more likely to present PCOS, respectively." And the sentence above for the discussion section, since the explanation for the reason why "That is, a higher plasma TMAO level or LH/FSH ratio increases the risk of PCOS." is exposed in this section (because TMAO and LH/FSH ratio were shown to be independent predictors of PCOS).

Are the methods appropriate and well described? If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls? If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown? If not, please explain in your comments to the authors.

Yes

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If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

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