Reviewer's report

Title: Metabolic characterization of menopause: cross-sectional and longitudinal evidence

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Reviewer: Elizabeth Want

Reviewer's report:

This manuscript describes the analysis of NMR generated metabolic profiles in order to investigate the effect of menopause on cardiometabolic biomarkers. This is an interesting subject which should be of interest to many in the field. The study involves the analysis of a large data set by experienced researchers. I have only a few minor comments below:

* Abstract. It is not immediately clear why these markers need to be studied. Perhaps there could be a sentence stating the increased risk of heart disease, etc. in post-menopausal women.

* Introduction. The Introduction is rather brief and so, if possible within the word limits, I would like to see details on the following points:
  o What are the cardiovascular risk factors? (Line 15)
  o What is meant by adverse levels? (Line 18)
  o Why was NMR used to obtain these metabolite profiles? Why not mass spectrometry?
  o Why serum samples?
  o Does NMR "miss" any metabolites that would be useful as biomarkers?
  o How were the 74 biomarkers chosen? Also, can they be called biomarkers at this stage? Are they not simply metabolites until they are found to be a biomarker of a specific disease/risk/state, etc?

* Methods. Did the authors consider performing different statistical tests on the data, e.g. univariate statistics on individual metabolites?

* Should the Bonferroni correction not be performed on the 74 metabolites rather than the 19 principal components? This is a conservative test - why not Benjamini-Hochberg?

* Table 1. It should say mothers/participants not mums.

* What effect does the age of menopause and/or the length of time taken to go from pre- to post menopause have on these metabolites and risk factors?
Similar to the point above, did the authors investigate the "rate of change" of these specific metabolites? Was there a more dramatic change (increase or decrease) in these metabolites if the woman underwent a quicker/earlier menopause?

There should be more discussion on confounders, e.g. education. How does education impact upon the results? Is it mainly dietary education, i.e. poor diet that is influencing BMI/cardiovascular risk factors?

Also, I would have liked more discussion on the specific metabolites found to be significant/of interest and the biological implications of these changes.

Why was the group on lipid lowering medication included? Surely this is a huge confounding factor?

What are the future plans?

- Would urine also be a useful biofluid to study?
- Have the authors thought about linking hormone and metabolite data?
- Are there plans to follow these women further to see whether they do develop cardiovascular disease events?

**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

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

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