Author’s response to reviews

Title: The 1H NMR Serum Metabolomics Response to a Two Meal Challenge: a Cross-Over Dietary Intervention Study in Healthy Human Volunteers.

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Answer to reviewers and editors comments.

Thank you for your relevant and discerning comments. We appreciate your work on the manuscript. Find rebuttal to each point below. Revisions in the manuscript are colored and also highlighted using track-changes.

Reviewer 1.

1. Time for test breakfast was from 7.30 up to 9.30 (Figure 1), but did participants have a specific range of time for completing their breakfast? If yes, how much time? Please add some information in the text.

Answer: No participants did not have a specific range of time for completing their breakfasts and we did not record the time between start and finish of breakfasts. However, participants were generally hungry in the morning and started work or lectures directly after breakfast why on an estimate it did not take longer than 15-20 minutes. We have now revised the text (line 177-179) to : Volunteers were instructed to drink water for the evening meal, not eat anything further and
only drink water before arriving to the test kitchen between 07.30 and 09.30 h where they consumed the breakfasts within 30 minutes.

2. They are instructed to follow some instruction for standardizing all procedures (lines 52-56; lines 1-4 of study design), did you check their compliance on the day before and then during the intervention? If yes, How?

Answer: We checked study participants’ compliance by asking them to every morning before intervention breakfast register on a form health status, occasional medications, and exact time of evening meal together with water intake during the overnight fast and asking if they had done something that diverged from the instructions.

3. Did you instruct participants for specific restriction at lunch? Please add

Answer: Study participants had restrictions regarding alcohol and fish intake during the intervention (line 169-173). We have now added (Line 173): Volunteers did not have any other restrictions regarding food consumption.

Minor considerations

Please change "equicaloric" with "isocaloric" through the text.

Answer: We have now changed equicaloric to isocaloric throughout the text.

Lines 45-46: This sentence is repetitive, please revise the paragraph

Answer: Lines 45-46 are part of Results in Abstract, where unique information is provided. Hence, we do not understand what this is referring to.

Materials and methods Lines 18-19: please add a reference or a description for the lifestyle questionnaire

Answer: The questionnaire used was a short lifestyle questionnaire developed in house in the project "Dietary intake in medical and nursing students 2007-2012". Master thesis in medicine, Programme in medicine, Sahlgrenska Academy, University of Gothenburg, Sweden, 2012. It included questions regarding alcohol and food consumption, use of nicotine, drugs, herbal remedies and supplements, allergies and level of physical activity.
Line 47: Could be useful to add at least the amount of energy and macronutrients for the two breakfasts.

Answer: The amount of energy is described in Line 160 and we have now also added the macronutrient content (Line 163-167): Breakfasts of 500 kcal comprised 20g protein, 19g fat and 60g carbohydrates while the breakfasts of 750 kcal comprised 29g protein, 34g fat and 80g carbohydrates…

Reviewer 2.

1. Title and abstract contain the abbreviation 1H NMR that is never explained. Other abbreviations in the abstract are never explained. Title includes a comma which should be a colon.

Answer: We have now changed the comma in the title to a colon. In addition, we have now explained NMR and other abbreviations in the abstract. Regarding the use of NMR in the title, in our field using NMR metabolomics the abbreviation is commonly used in the title. We leave this to the Editor to decide.

2. Abstract does not represent the manuscript and should be revised substantially to reflect the background (ie what gap needs to be filled by research) the aim of the work and what was actually done in the manuscript.

Answer: We have now rewritten the abstract to better reflect the content of the manuscript

3. Double-spacing text and using descriptive content headings rather than numbers would be preferable, as would continuous line numbering and less abbreviations. Some of the tables are all abbreviations.

Answer: The text is now double-spaced and line numbering has been added. We have also removed the numbering of headings and more descriptive headings. Regarding abbreviations, we have now checked so they are properly described in the manuscript.

4. The background section should introduce us to the terms that will be used throughout the manuscript and provide a rationale for your methods. The current background should be condensed into 1-2 paragraphs that explains the gaps in the field of measuring diet exposure, and then a paragraph that introduces metabolomics (and what it can measure, amino acids etc) and the different types of models/statistical techniques or at least that statistical techniques need to be developed/tested. Then your aim and methods will follow nicely.
Answer: The Background is now condensed into two paragraphs focusing on current difficulties in measuring dietary exposure and an introduction to metabolomics and the rationale for methods used in our study.

5. The methods section could do with more references, so that the reader can understand that there is a valid rationale for the methods you chose.

Answer: We have now added more references in the methods section to show the rationale for methods used. References 22,23,25,26 and 28.

6. Why were participants not allowed to eat fish the day before?

Answer: At the time, when the intervention was conducted, there were some reports about fish having a complex influence on the metabolome i.e. levels of Trimethylamine-N-oxide (TMAO) why we choose to exclude fish from participants diet the day before the intervention as a precaution.

7. The discussion section confused me. I though the point of the study was to test how well the models identified the dietary exposure, so I do not understand why the authors explained their amino acid findings… I thought those were expected based on the foods eaten? I thought that was the point? That the researchers gave 2 different breakfasts and then used the metabolomics biomarkers to identify which foods the participants had been given.

Answer: The aim of the study was to see if we could identify metabolic profiles from two different breakfast meals and how well the different models performed regarding reproducibility and discriminative potential of metabolic profiles and biological relevance of selected discriminating metabolites. Breakfasts were isocaloric but consisted of different foods. We wanted to see if we could identify metabolites connected to the consumption of these foods 3h postprandial. This is why we write about the different metabolites, to give a biological understanding of the increased levels of metabolites, in relation to the foods consumed. The metabolites could be different either because of the food content or due to changes in the metabolism.

8. After reading the discussion section, I am not clear what model and methods the authors would recommend.

Answer: We have rewritten the conclusion section and hope this will clarify how the results differ between the statistical models inquired.

9. I am also left not understanding the implications or future directions of this research after reading the paper. This is a topic that I would like to know more about and that could potentially
help the field of nutrition, but the language and structure of the manuscript is unusual and I find it difficult to follow.

Answer: With revisions made throughout the manuscript especially in the Background and Conclusion sections we have tried to clarify the potential use for the field of nutrition and future directions regarding implications of different multivariate models for dependent samples.

Associate Editor

1. Some additional references to be added to the methods section so that readers can understand why you used each method and learn more about the methodology involved in the application of metabolomics to the field of nutrition.

Answer: We have now added more references in the methods section to show the rationale for methods used. References 22,23,25,26 and 28.

2. Revision of the background to include a clear introduction to the terminology and rationale behind your project (ie what specific 'gap' does your project propose to fill?)

Answer: The Background is now condensed into two paragraphs focusing on current difficulties in measuring dietary exposure and an introduction to metabolomics and the rationale for methods used in our study.

3. Revision of the discussion and conclusion to include a clear description of the new knowledge/information that your project has provided.

Answer: We have rewritten the conclusion section and hope this will clarify how the results differ between the statistical models inquired.