

Lipidomic analyses of large cohort studies define the role of lipid metabolism in bridging diet and cardio-metabolic health

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This manuscript has been previously reviewed at another journal. This document only contains information relating to versions considered at Nature Communications.

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

I appreciate the work the authors have put into improving the clarity of the manuscript. However, I have few remaining points:

1. Lipid scores can replace dietary assessment through questionnaires, but I do not agree that these help in giving personalized feedback in the absence of questionnaire data. What will a person change if they have a low score in MIND? You need to know which foods are currently consumed by that person to then know, what needs to be changed to improve the score. Without the specific info on intake, the level of feedback does not go beyond any general dietary advice. I would severely tone down any reference to personalization potential.

2. I find the superior risk association of the lipid-based scores over the questionnaire-based indicative of the fact that the scores not only represent dietary exposure, but also metabolic health status. This fits with the observation that they only predict small fractions of the diet scores in the testing sample (supplement fig 8 to my understanding, please also check if referencing is correct here). My question remains, how useful that is. The lipidomics scores are not faithful to the original score nor are they optimized to predict disease risk.

3. Regression models with several predictors should be correctly termed "multivariable" not "multivariate". Also "univariate" refers to one dependent variable and not models including only one predictor. Please adapt.

Reviewer #2

(Remarks to the Author)

I would like to thank the authors for addressing my comments in detail. The only minor comment I have is to add "with" between "associated" and "circulating" in line 281.

Reviewer #3

(Remarks to the Author)

NCOMMS-25-64840-T

In this article, the authors conducted large-scale lipidomic analyses with comprehensive statistical analysis to assess the associations between diet and cardiometabolic health. Here are some points the authors may consider to improve the manuscript.

Major:

1. Table 1: DGI, HSR-DI, and MIND scores were not available for MCCS subcohort. What hindered the calculation of such scores for this validation subcohort?

2. Table 2: What would be the implications of the findings from model 2? I think it would be nice to elaborate the findings in the discussion. Also, it seems that the lipidome-based signatures did not perform substantially better than FFQ-based

signatures. Could the authors elaborate if this finding was expected, and if it was worthed to perform the most costly lipidomics analysis which may not outperform the FFQ in predicting mortality risk?

3. Page 22 line 701-702: since the lipid panels covered by both targeted methods were not identical, did the subsequent analysis include only lipids covered by both methods? Also is there any difference in the sensitivity or in the quantity measured by both methods?

4. Considering that large-scale omics analysis is usually performed to generate hypotheses, what would be the hypotheses generated by these findings which may be relevant or interesting to be tested in future studies? Could the authors highlight some specific molecular mechanisms or regulatory pathways relevant to the findings?

5. The utilized dietary indices have large overlapping dietary components, as evidenced by high correlations across the lipidomic profiles (Supplementary Figure 7). Could the authors elaborate on the potentially shared dietary components across such indices responsible for the high correlations?

Minor:

1. Page 20 line 699: MRM transition was found in Suppl Table 15 not 14.

2. Could the authors elaborate more on the standardization method applied on dietary predictors and the cardiometabolic health markers?

3. Figure 2: the panels are named abc and there are also footnotes (abc) about elution order. Perhaps the authors could consider using different markings to avoid confusion?

4. Fig 3: what are panels a, b, and c?

5. More findings were mentioned to be listed on metabolomics.baker.edu.au/DietLipidomeXplorer/, but username and password would be required to sign in. Perhaps the authors would need to make it clear that the findings are not publicly available?

Reviewer #4

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

I have no further comments on this manuscript and appreciate how my previous suggestions have been addressed.

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REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

I appreciate the work the authors have put into improving the clarity of the manuscript. However, I have few remaining points:

1. Lipid scores can replace dietary assessment through questionnaires, but I do not agree that these help in giving personalized feedback in the absence of questionnaire data. What will a person change if they have a low score in MIND? You need to know which foods are currently consumed by that person to then know, what needs to be changed to improve the score. Without the specific info on intake, the level of feedback does not go beyond any general dietary advice. I would severely tone down any reference to personalization potential.

Response: We agree with the reviewer, the lipid scores do not replace the diet, rather we see the lipid scores as complementary biomarkers that can objectively capture the metabolic imprint of diet quality, overcoming some of the limitations of self-reported data. We have now toned down the language regarding “personalization potential.” Specifically, we emphasize that while lipid-based scores may serve as biomarkers of overall dietary quality and metabolic health, actionable feedback for individuals would still require integration with traditional dietary assessment data. Page 19, line 677-681.

2. I find the superior risk association of the lipid-based scores over the questionnaire-based indicative of the fact that the scores not only represent dietary exposure, but also metabolic health status. This fits with the observation that they only predict small fractions of the diet scores in the testing sample (supplement fig 8 to my understanding, please also check if referencing is correct here). My question remains, how useful that is. The lipidomics scores are not faithful to the original score nor are they optimized to predict disease risk.

Response: Indeed, the lipid-based scores capture only a modest proportion of variance in the questionnaire-derived diet scores (as shown in **Supplementary Figure 8**, which is correctly referenced). The lipidomics-derived scores appear to integrate both dietary exposure and the downstream metabolic response, effectively reflecting how an individual’s biology translates dietary patterns into metabolic outcomes.

While they are not intended to be direct surrogates of the questionnaire-based diet indices, their stronger associations with disease risk suggest that they capture biologically relevant variation beyond self-reported intake. In other words, they provide a more functional, phenotype-oriented readout of diet quality, one that bridges nutritional exposure with metabolic health status. Therefore, their utility lies in providing additional information and offering a metabolically anchored tool that may enhance disease risk assessment and mechanistic understanding of diet–health relationships. Page 18 line 603-611 and page 19 line 677-681.

3. Regression models with several predictors should be correctly termed “multivariable” not “multivariate”. Also “univariate” refers to one dependent variable and not models including only one predictor. Please adapt.

Response: We have revised the terminology throughout the manuscript to use “multivariable” for models with multiple predictors and “univariate” only to refer to analyses with a single outcome.

Reviewer #2 (Remarks to the Author):

I would like to thank the authors for addressing my comments in detail. The only minor comment I have is to add "with" between "associated" and "circulating" in line 281.

Response: We have added this. Line 281.

Reviewer #3 (Remarks to the Author):

NCOMMS-25-64840-T

In this article, the authors conducted large-scale lipidomic analyses with comprehensive statistical analysis to assess the associations between diet and cardiometabolic health. Here are some points the authors may consider to improve the manuscript.

Major:

1. Table 1: DGI, HSR-DI, and MIND scores were not available for MCCS subcohort. What hindered the calculation of such scores for this validation subcohort?

Response: We included all seven diet indices in AusDiab because these measures were already available. In contrast, for the MCCS cohort, we did not have access to the same codes for specific scoring algorithms used to derive DGI, HSR-DI, and MIND in AusDiab. Given this limitation, and to ensure consistency and reliability in the validation process, we focused on validating the three scores for which complete and comparable information was available. We have noted this in the manuscript for transparency (Page 13 line 401-402).

2. Table 2: What would be the implications of the findings from model 2? I think it would be nice to elaborate the findings in the discussion. Also, it seems that the lipidome-based signatures did not perform substantially better than FFQ-based signatures. Could the authors elaborate if this finding was expected, and if it was worthed to perform the most costly lipidomics analysis which may not outperform the FFQ in predicting mortality risk?

Response: Model 2 includes all covariates from Model 1, with the addition of the diet score and its corresponding metabolic (lipidome based) signature. This allows us to

determine whether the metabolic signature provides information independent of the diet score itself. As such, in Model 2, the lipidome-based signatures outperformed the FFQ-derived diet scores (as shown by the p-values in the last column). All lipidome-based signatures except those corresponding to the LCD score and HSR-DI were significantly associated with mortality risk, even after accounting for the diet scores themselves. Indeed, we have elaborated these in the manuscript page 15 line 473-476 and page 18, line 603-611. Further details on Model 1 and Model 2 can be found in the footnote too (Page 33, line 1290 and 1292).

3. Page 22 line 701-702: since the lipid panels covered by both targeted methods were not identical, did the subsequent analysis include only lipids covered by both methods? Also is there any difference in the sensitivity or in the quantity measured by both methods?

Response: The analyses include the lipids common to both cohorts for replication purposes. We have utilised a standardised reference material (NIST SRM 1950 plasma) used internationally for harmonisation of reported values for each lipid species (ref 121).

While there are slight sensitivity differences between instrumentation generations (Agilent 6490, vs Agilent 6495C), only lipids detectable on both cohort studies were include in this analysis Page 21, line 795-797.

4. Considering that large-scale omics analysis is usually performed to generate hypotheses, what would be the hypotheses generated by these findings which may be relevant or interesting to be tested in future studies? Could the authors highlight some specific molecular mechanisms or regulatory pathways relevant to the findings?

Response: Thank you for this thoughtful question. In the present analysis, we define comprehensive diet-related lipidomic signatures in the largest cohort studied to date and demonstrate that these signatures better predict cardiometabolic outcomes than the diet derived scores and mediate the effect of diet on all-cause mortality. This framework will in future, provide a tool to calculate many different diet scores and assess their effect (in silico) on cardiometabolic outcomes to pave the way for personalized nutrition strategies to prevent cardiometabolic disease (page 18 line 632-637).

We have also highlighted some of the potential molecular mechanisms or regulatory pathways relevant to some of the major diet signatures such as dairy (page 16, line 528-537), red meat (page 16 lines 542-544), fish intake (page 17 lines 565-570), alcohol related signatures including the specific enrichment of lipid species such as 16:1, which are well-recognised markers of de novo lipogenesis and often elevated in the context of alcohol intake, suggests a separate hypothesis: that alcohol-related metabolic pathways, including increased hepatic lipogenesis and altered SREBP-

1c/FAS regulation, may contribute to the observed risk profiles (page 17 line 582-586, and lines 592-597). Also page 18 lines 653-658 elaborates need for future studies involving stable-isotope feeding trials, lipid-tracer studies, and functional studies in cell and animal models to map the metabolic pathways involved. Exploring these pathways in future studies in controlled trials may clarify the causal links between dietary exposures, lipidomic responses, and long-term health outcomes.

5. The utilized dietary indices have large overlapping dietary components, as evidenced by high correlations across the lipidomic profiles (Supplementary Figure 7). Could the authors elaborate on the potentially shared dietary components across such indices responsible for the high correlations?

Response: Yes indeed. Diet quality scores in particular HSR-DI, DGI, MIND, GDQS and Mediterranean diet all assign higher scores to higher intakes of nutrient-dense foods such as fruits, vegetables, whole grains, legumes, fish and healthy fats and lower intakes of refined grains, added sugars, and processed foods. Because these shared components influence similar metabolic pathways, they produce highly overlapping lipidomic signatures across the different indices (Supplementary Figure 7). There is a high correlation across the diet scores themselves (Supplementary Figure 6). We have included this elaboration in the manuscript discussion (page 11, line 363-368).

Minor:

1. Page 20 line 699: MRM transition was found in Suppl Table 15 not 14.

Response: This is fixed (now Supplementary Table 16 in the revised manuscript).

2. Could the authors elaborate more on the standardization method applied on dietary predictors and the cardiometabolic health markers?

Response: We have elaborated the standardisation method (page 23 line 870-872).

3. Figure 2: the panels are named abc and there are also footnotes (abc) about elution order. Perhaps the authors could consider using different markings to avoid confusion?

Response: We have differentiated these into capital later for the figure legends (page 6 Figure 2).

4. Fig 3: what are panels a, b, and c?

Response: We have labelled these A) dairy, B) red meat and C) fish (Page 8 figure 3).

5. More findings were mentioned to be listed on metabolomics.baker.edu.au/DietLipidomeXplorer/, but username and password would be required to sign in. Perhaps the authors would need to make it clear that the findings are not publicly available?

Response: The website currently requires login because it is not yet publicly accessible (this was initially sent in the letter to the editor for reviewers). The username and password (username: **reviewer**, password: **6495cLipidomics**) is accessible for reviewers (this has been provided to the editor). Once the manuscript is published, the materials will be made publicly available.

Reviewer #4 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.