nature portfolio

# Peer Review File

## Systems biology approaches identify metabolic signatures of dietary lifespan and healthspan across species

**Editorial Note:** This manuscript has been previously reviewed at another journal that is not operating a transparent peer review scheme. This document only contains reviewer comments and rebuttal letters for versions considered at *Nature Communications*.

**REVIEWERS' COMMENTS**

Reviewer #1 (Remarks to the Author):

I commend the authors on thoroughly updating their Mendelian randomization reporting of the methods, the updated analysis, and revised interpretations, following my comments in the previous submission. Thank you for the thorough response. I recommend the article be accepted.

Reviewer #1 (Remarks on code availability):

I only partially reviewed the R code but appropriate packages are loaded and functions appear to be used and interpreted correctly, in line with the resports in the manuscript.

Reviewer #4
The study identified metabolites that influenced responses to dietary restriction in drosphila using the DGRP, then attempted to cross validate the drosophila results using mendelian randomization in human data from the UK Biobank. Orotate and threonine were identified as metabolites that were either harmful or beneficial respectively to the outcomes of dietary restriction (lifespan in drosophila) or aging in humans (parental age at death). The manuscript has been amended in detail in response to a genetics and statistical review. The responses have largely addressed these comments.

Minor comments
What are the ages of the UK Biobank participants? Do the participants' data reflect human aging more generally.
Line 59 human genetic studies of longevity have robustly found associations with APOE and FOXO3 variants, these should be mentioned.
Reference 13 is incorrect (and many references need to be re-formatted)
Machine learning is becoming routine so the comments in the introduction about its utility could be removed (line 67)
Are there human UK Biobank data on food intake ie the drosophila data are the response to dietary restriction? This could be used to analyze human data on age-related phenotypes in response to different diets that might more closely reflect drosophila responses to dietary restriction
The drosophila database used dietary restriction by dilution. The authors might consider the results of Liu et al (Speakman Cell Metab 2022) on the comparison between genetic mechanisms for the differing effects of restriction by dilution versus standard dietary restriction.
The study of mice and genetic response to caloric restriction (Liao et al Aging Cell 2010) and a statistical critique is relevant to the discussion (Senior J Gerontol A Biol Sci 2023) could be discussed.
There is no discussion of the limitations of the study.

**Reviewer #4 comments and responses:**
The study identified metabolites that influenced responses to dietary restriction in drosphila using the DGRP, then attempted to cross validate the drosophila results using mendelian randomization in human data from the UK Biobank. Orotate and threonine were identified as metabolites that were either harmful or beneficial respectively to the outcomes of dietary restriction (lifespan in drosophila) or aging in humans (parental age at death). The manuscript has been amended in detail in response to a genetics and statistical review. The responses have largely addressed these comments.

Minor comments
What are the ages of the UK Biobank participants? Do the participants' data reflect human aging more generally.

The core UK Biobank cohort included participants aged 40-69 in 2006-2010. Our study included data used to identify associations with metabolites collected from individuals spanning 32 to 87 years of age, with 3 timepoints taken for each individual. Data from Shin, et. al. 2014 includes data collected from participants ranging from 17-102 years of age. One set of GWAS data used in our MR analysis, which identified various genes associated with parental age at death from participants in the UK Biobank, included people aged 40-73 with parents ranging from 46-107 years of age. Further detailed information on the participants can be found in the original publications.

The participants' data included in the UK Biobank characterizes the aging in 500,000 middle-aged to older individuals and would likely reflect general human aging from midlife onward, though it would probably be most applicable to individuals of European descent and would limit the generalizability of our specific findings to other populations. However, the UK Biobank data captures the variation seen across individuals of similar descent, which served as an appropriate comparison for our fly dataset using multiple genotypes. These broader implications of our work would potentially be more generalizable to non-European populations.
Line 59 human genetic studies of longevity have robustly found associations with APOE and FOXO3 variants, these should be mentioned.

Added the following sentence to the introduction, "Despite this overall disconnect, there are genes that have repeatedly shown an association with lifespan, such as FOXO3 and APOE8,9."
Reference 13 is incorrect (and many references need to be re-formatted)

Corrected reference 13 and other duplicate references. We thank the reviewer for pointing out the oversight.
Machine learning is becoming routine so the comments in the introduction about its utility could be removed (line 67)

Removed first sentence of this paragraph, "Recent advancements in analytical techniques have enabled more comprehensive investigations into intricate traits."**.**
Are there human UK Biobank data on food intake ie the drosophila data are the response to dietary restriction? This could be used to analyze human data on age-related phenotypes in response to different diets that might more closely reflect drosophila responses to dietary restriction

Thank you for your insightful suggestion. The UK Biobank does contain extensive data on food intake, collected through a touchscreen questionnaire and a web-based 24-hour dietary

assessment tool. These data have been linked to various health outcomes, providing a valuable resource for future comparisons with our Drosophila dietary restriction studies.

While our current focus is on metabolites as potential modulators of age-related responses, we appreciate the idea of exploring human dietary patterns that mirror Drosophila responses. This comparison could enhance the translational aspects of our research, and we will consider it in future studies.

The drosophila database used dietary restriction by dilution. The authors might consider the results of Liu et al (Speakman Cell Metab 2022) on the comparison between genetic mechanisms for the differing effects of restriction by dilution versus standard dietary restriction.

We thank the reviewer for suggesting that we compare the mechanisms between calorie restriction and calorie dilution. A study from the Tatar group (Min Exp Gerontol 2007) showed a caloric content-dependent lifespan in flies. We would surmise a genotype-specific response to two different methods of reducing calories, and that the mechanisms would be different, but have some overlap.

The study of mice and genetic response to caloric restriction (Liao et al Aging Cell 2010) and a statistical critique is relevant to the discussion (Senior J Gerontol A Biol Sci 2023) could be discussed.

The critique of the Liao study, and other studies with low numbers of animals per genotype, highlights the need for high N per genotype. The fly data set we used in our study included at least 200 flies per genotype for DGRP lines. Further, our validation experiments included at least 100 per group. At these N values, the data variance corrected for by partial pooling should be unnecessary, as shown in the Senior study at N values above 40.

There is no discussion of the limitations of the study.

Added, “Despite these limitations, the broader implications of our work would potentially be more generalizable to other populations and highlight the need to study individual-specific responses in lifespan.” To the discussion following a description of limitations of our study.