

Author's response to reviews

Title: Red wine consumption increases antioxidant status and decreases oxidative stress in the circulation of both young and old humans

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Author's response to reviews:

Dear Dr Kumagai

Please find attached the revised version of the manuscript (MS: 1533966001126051) we hope to have published in the Nutrition Journal. The comments of both reviewers have been taken into consideration and a number of alterations to the previous version of the manuscript have been made, these changes and responses to the reviewers' comments are detailed below.

Reviewer 1

1. The analysis of glutathione, malondialdehyde, total antioxidant status, serum glucose and plasma lipid was not described in detail as how the analysis was done using a commercial kit, such as how much sample was added, how the result was obtained, and so on. It is suggested that the method part involving the use of the commercial kit was written in more detail, so that the reader can understand the method better.

Response: More detail on each of these methods has been included in the relevant methods sections of the manuscript.

2. Page 13, Table 2 indicated in the text was not found. Please correct the number of the listed Tables and Figures.

Response: This oversight has been corrected; all tables and figures are now correctly labelled.

3. What are the two-factors used for the two-way analysis? It seems that the statistics analysis was three-way (young versus old, with and without consumption of wine at the same time, and before and after consumption of wine for each group), not two-way analysis, as shown in figure 2-4. For example, it was not which group was the control group data compared to? It is suggested that the part of "statistical analysis" on page 8 be written

in more detail.

Response: Upon revision it was agreed that a three way ANOVA would be a more appropriate method to analyse the data in figures 2 - 4. However the authors feel that including the results of this additional analysis for all data would detract from the key finding of the study. I.e. the effect of red wine consumption was the primary objective of the study not the effect of time on baseline (pre) values. In addition the cross over design used in the study enabled the effect of red wine to be more clearly identified irrespective of baseline values. Additional information relating to this interpretation of data has been included in the statistical analysis section of the manuscript.

Reviewer 2

1.The title does not represent well the main conclusion. I suggest to state the different behaviour after wine intake of young and elderly people or in normal antioxidant situation and with augmented oxidative stress due to aging.

Response: The titled has been changed to be more representative; it now reads 'Red wine consumption increases antioxidant status and decreases oxidative stress in the circulation of both young and old humans.

2.Wine's chemical composition has to be analysed. At this point in the state of the art of this topic one has to know which compounds are volunteers taking. This is also useful to compare data from different authors. A table with phenolic concentration is required.

Response: The composition of the wine has been analysed by the Australian Wine Research Institute, an NATA accredited analysis laboratory. The results of this analysis have been used to construct a new table which summaries the wines composition.

3.Page 3 Line 5 Reference 2 is not adequate to mention a study of radical ability scavenging after the up regulation of the enzymes. Move it to the end of the paragraph.

Response: As suggested the reference has been moved to the end of the paragraph.

4.Did they control the diet in foods containing large amounts of phenolic compounds such as berries, onion, chocolate, tea?

There were no specific instructions given to avoid foods containing large amounts of phenolic compounds, other than abstain from consuming any alcohol, grapes or grape products.

Response: This information has been included in the revised manuscript in the Intervention design

5. Page 8 Did they checked the normality of their data? In case they did, please state. If data do not follow a normal distribution, just apply a non parametric test.

Response: Data was checked for normality and the fact that it was normally distributed has been included in the statistical analysis.

6. The first paragraph in the discussion section is more like for an introduction section

Response: Upon revision it was agreed that the first paragraph of the discussion needed to be restructured, this has been done.

7. Page 11 Conflicting evidence... Please verify that reference 17 uses plasma for the determination of plasma antioxidant activity. Indeed authors compared plasma and serum values revealing lower values for serum than for plasma.

Response: Revision of the reference 17 confirms that plasma was used in the study cited. As a result it was felt that comments on the validity of plasma versus serum measurements of antioxidant status be removed from the manuscript.

8. Page 12 Sentence Our results show that the effect.....could be related to the elevation of polyphenol concentration in plasma. This is only speculation because they did not determine any phenolic compound.

Response: We agree that this sentence was speculative as the polyphenol content was not measured in plasma. The sentence has been removed from the manuscript.