Author's response to reviews

Title: Supplementation of a western diet with golden kiwifruits (Actinidia chinensis var.'Hort 16A':) effects on biomarkers of oxidation damage and antioxidant protection

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Version: 2 Date: 28 April 2011

Author's response to reviews: see over
Dear Editor-in-Chief

Please find attached our revised manuscript entitled “Supplementation of a western diet with gold kiwifruits (Actinidia chinensis var.‘Hort 16A’:) effects on biomarkers of oxidation damage and antioxidant protection”, to be considered for publication in Nutrition Journal.

In the following pages we have tried to answer the questions and concerns expressed by the reviewers. Reviewer no 1 and 2 seemed to have more general comments rather than specific questions. We have focused on the specific questions of reviewer no 3, assuming that the general response from reviewer no 1 and 2 also was referring to and included the specific questions of reviewer no 3. Our response is highlighted in bold text.

Track changes has been used to highlight changes in the manuscript. We have not used track changes on the tables though, but they have also been adjusted to comply with the suggestions expressed by the reviewers.

Sincerely,

Asgeir Brevik

Asgeir Brevik
Reviewer: Patrizia Riso
Reviewer's report:

• Major Compulsory Revisions

1- Briefly, in this manuscript the authors have reported the results obtained in an intervention study with two different portions of gold kiwi fruit (1 or 2 fruits) with the aim to verify whether supplementation for 4 weeks with this fruit could affect several markers of oxidative damage and antioxidant protection. On the whole the study is very interesting and add some new results on the protective role of fruit intake. However, despite the experimental design involving a cross over intervention is appropriate, it lacks of a control treatment and this should be mentioned as a possible limit of the study (see later in the discussion).

While the number of participants in this study could have been better we believe the cross-over study is a strong design with no need for additional control groups. The whole point of doing a cross-over is that subjects are their own controls, the design is supposed to be robust and less affected by high and low responders. On the other hand it would be nice to study seasonal variation in the results from a control group followed for a whole year. Given our climate and latitude there would probably be some fluctuations over the course of a whole year. We have added a paragraph suggesting a control group in the discussion of possible limitations.

2- Statistical analysis
The Student t-test used is appropriate to test differences before and after a given treatment. However ANOVA for repeated measure design, with the treatment (1 and 2 kiwifruits) and the time (before and after treatments) as factors can allow the authors to ascertain whether the amount of fruit added to the diet is a significant factor in the modulation of the effects observed.

We believe the Anova approach could be questioned in our case. The main problem, as we see it, is that our study is a cross-over and that we have one sample from all the subjects on one kiwifruit, one sample from all on two kiwifruits, and two samples from all subjects on zero kiwifruits (baseline). In other words, we have two baselines from each subject and it is not obvious which one of them should be used in the model (we don’t think we can have both in there at the same time and we have absolutely no reason to choose one over the other). That is why it could be argued that the Anova approach is not ideal in our case (we don’t feel comfortable with this approach).

3- Statistical analysis
Please add to this paragraph that simple regression analysis was performed to evaluate correlation between plasma vitamin C and strandbreaks and report such data in the result section.

The suggested paragraph has now been added and all results have been moved to the results section.

4 – Discussion
4.1 - I suggest to revise the first statement by underlying only significant results and eventually stating trends.

In the revised manuscript we have tried to be more specific in the first statement of the discussion with respect to indication of significant observations.

4.2 - The low increase observed in vitamin C levels following the intake of one kiwi fruit is presumably the effect of the good initial vitamin status of the group of subjects considered (vitamin C concentration increase more in deprived subjects even when low amounts of vitamin are introduced). The fact that higher vitamin levels can be found following acute intakes is not essential for this “long term” study. On the contrary it is interesting that 2 kiwifruits can significantly increase plasma vitamin C levels (even if, as previously reported, it would be useful to know the amount of vitamin introduced through the fruits).

Information on vitamin C content of kiwifruits has been added to the introduction.

4.3 - As regard the statement on the better effect of yellow with respect to green kiwifruit in improving resistance towards H2O2 oxidation, it should be added: “even if a direct comparison cannot be performed”

This subordinate clause has now been added in the revised manuscript.

4.4 - I do not completely agree with the discussion on “active ingredients in kiwi fruit” and the concept of “saturation effect”, in fact as the authors also mentioned, in the case of whole foods, many different compounds can act in synergy and this may also explain the lack of a dose response. It may be the authors can add it as a possible explanation.

This comment is a little difficult to understand. We are not sure how synergy effects can explain the lack of dose-response if we completely disregard all types of saturation.

4.5 - As regard correlation between biomarker of DNA damage and plasma vitamin C. Again you cannot be sure that the correlation with just one active compound is sufficient to describe a phenomenon and declare that the effect is not dependent on antioxidant action …in other terms, you cannot exclude that there is a concerted action of many compounds. For example what about phenolic compounds or folates, do the authors know whether golden kiwifruit is a good source?

This is a valid concern and the statement has been modified accordingly in the revised manuscript.

4.6 - While the choice of a cross over design for this study is optimal and I trust most of the results obtained, one possible limitation is the absence of a control treatment (i.e. a treatment without kiwifruit) only in this way it would have been possible to exclude that results have been obtained by chance. This limit need to
be included in the last part of the discussion.

The possible benefits of adding a control group has been mentioned in the last part of the discussion in the revised manuscript.

We have could have

• Minor Essential Revisions
  1- Measurement of plasma vitamin C
  The description provided does not allow to understand how the authors analyze both reduced and total ascorbic acid concentration in plasma. Please add a short description of the procedure used (as reported in the cited article).

  We have added some more info to the description. In addition there is a reference to a paper in which the method has been described in detail.

  2 - Measurement of plasma carotenoids
  It is not stated the amount of plasma extracted for such an analysis. Please add it.

  Volume has been added.

  3- Measurement of MDA
  Please, complete the characteristics of column and add the volume of injection.

  The requested information has been added in the revised manuscript.

  4- Results
  The authors wrote: “No differences in the expression……before the kiwifruit consumption period and the period without kiwifruit”. This statement seems not correct. You did not report a control period, it may be you should write before and after the kiwifruit consumption.

  True. This sentence has now been changed.

  5- Tables
  In general it is suggested to avoid two decimals for variables considered such as vitamin C and FRAP values. Moreover the term “diff” is not clear you should state what you mean (e.g. % changes …..) and SEM should be also reported. It should be also calculated as (after the treatment – before the treatment/ before the treatment *100) in this way you have for example that vitamin C increase by 4% no decrease as now reported.

  All tables have been updated in the revised manuscript.

• Discretionary Revisions
  1 - Abstract
I suggest to modify the conclusion as follow: Golden kiwifruit consumption strengthen (or improve) resistance towards endogenous and exogenous oxidative damage.

Ok. The sentence has been modified.

2- Introduction
The last paragraph reports a brief statement on results obtained and consideration on green and gold kiwifruit. I suggest to move it in the discussion

We have chosen to leave this paragraph at the end of the intro as a brief summary.

3- No specific mention is present on the vitamin C concentration in the kiwifruits. Although it is clear that a certain variability is obvious in fresh fruits, at least a mean value should be provided in order to estimate the amount of vitamin C regularly added to the diet. This also allows a better discussion of results obtained at plasma level. I think it should not be difficult to find such data.

Waiting for reply
Information on vitamin content of kiwifruit has been included in the new introduction.

4 - The lack of effect of dietary intervention on DNA repair and expression of related genes is not unusual. We also found similar results in a study with broccoli intervention (please add to references)

The interesting observation from the new broccoli supplementation trial has been included in the new discussion.

5 - In the conclusion I would speak about “effect of regular intake of golden kiwifruit” instead of “supplementation”.

Excellent point. The conclusion has been changed to comply with the suggestion.

Level of interest: An article of outstanding merit and interest in its field