Author's response to reviews

Title: Polyphenol intake and mortality risk: an observational study within the PREDIMED trial

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Author's response to reviews: see over
Response to reviewers

DATE: March 17th, 2014

MANUSCRIPT: Polyphenol intake and mortality risk: an observational study within the PREDIMED trial

Ref. No.: 5954134271193566

Version 1

Reviewers' comments:

Reviewer: Augusto Di Castelnuovo.

Major Compulsory Revisions

1) The authors must report why the trial has stopped

Response: Thank you for the observation. We added a sentence and a reference to explain the reason why the trial was stopped: “The trial was stopped after a median follow-up of 4.8 years due to the benefit of the Mediterranean diets with respect to major cardiovascular events: myocardial infarction, stroke, or death from cardiovascular causes (analysis performed by the Drug and Safety Monitoring Board of the trial) compared to the control group [2],” on lines 131-135.

2) The authors stated that “The FFQ was validated to assess total polyphenol intake using total polyphenol excretion in 137 spot urine samples in a clinical trial (r=0.48, P<0.01) and in a cross-sectional study (r=0.26, P=0.04) [13]” Besides the statistical significance, these data does not convince this referee about the validity of the polyphenol intake assessment and the FFQ. Please clarify. The relatively low values of r should be commented at least as a limitation of the study.

Response: We appreciate the comment and we have included this information as a limitation of the study (lines 328-329).

3) The authors have to recognize as a limitation of their study the fact that the intake of polyphenols was not directly measured but only indirectly derived from a FFQ.

Response: We also agree with the comment and, accordingly, we have also included this statement as another limitation of the study (lines 326-327).

Minor Essential Revisions
Response to reviewers

4) The authors "noted that those who did not drink alcohol had a stronger inverse association for total polyphenol intake (HR 0.39, 95% CI, 0.17-0.90, P-trend=0.04) than drinkers (HR 0.99, 95% CI, 0.59-1.65, P-trend=0.91), but the interaction was not significant (P-interaction=0.16)". I agree with the decision of the authors to not emphasize this finding because of absence of statistical significance for the interaction term. However, the observed trend is suggestive, and of very interest if it will be established in other studies. I suggest to add a comment on it in the discussion.

Response: Thank you. That is a very good suggestion and we have added a paragraph in the discussion section (lines 277-280).

5) Please, add in table 1 the min-max values for total polyphenol intake at baseline in each quintile

Response: When adjusting for calories, we obtained estimated values for polyphenol intake (values are calculated using a regression curve). Because of that, some of these intakes got irrational values such as negative values. To solve this, it is a common practice to add a constant so the mean after adjustment gets the same value that the mean before adjustment. Therefore, we prefer to add cutoff points instead of the min-max values.

6) Polyphenols and other food and nutrient intake were adjusted for total calories using the residual method before entering in the survival analyses. However, the authors added the total energy intake in their multivariable models. I have some concerns about this approach. Please, clarify.

Response: Thank you for the comment; we understand this approach can lead to confusion. We adjusted intakes of polyphenols and nutrients by calories to compare relative values instead of absolute values. For instance, an intake of 1000 mg of polyphenols per day is not the same in a 3000 kcal-diet and in a 1500 kcal-diet. Polyphenols adjusted per calories allow performing better comparisons between groups.

On the other hand, mean calorie intake differed significantly between quintiles of polyphenol intake, and high calorie diets have been associated with high mortality risk so it seems logical to add it in the multivariable model.

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests: I declare that I have no competing interests
Reviewer: Shoichiro Tsugane

The authors investigated the association between polyphenol intake and total mortality using data from a randomized controlled feeding trial to assess the effects of Mediterranean Diet on primary prevention of cardiovascular disease among elderly men and women at high cardiovascular risk. They found an L shaped association between total polyphenol intake and total mortality. The inverse association persisted for stilbenes and lignans among the subclasses. The manuscript is methodologically sound and generally well-written. This reviewer has several comments as follows.

**Major Compulsory Revisions:**

1. Although the authors emphasized a long follow-up, an approximately 4.8 year follow-up is relatively short to assess a mortality endpoint and is subject to a reverse causation. The ill-health conditions leading to death may influence diet. Therefore sensitivity analysis excluding subjects who died during the first one- or two- year follow-ups should be done and described in the manuscript. Alternatively dietary data of one or two years before death may be excluded.

*Response:* Thank you. We agree with this comment and we have performed the sensitivity analyses suggested and the results have been added in the manuscript (lines 197-199 and 235-239).

2. Although the number of deaths was not sufficient to conduct a cause-specific analysis, the authors should at least show the finding on cardiovascular mortality. Moreover, the major causes of death should be shown.

*Response:* We recently published a paper about polyphenols and cardiovascular events (Tresserra-Rimbau, A; et al. Inverse association between habitual polyphenol intake and incidence of cardiovascular events in the PREDIMED study. Nutr Metab Cardiovasc Dis. 2014 Jan 22. [Epub ahead of print]).

A sentence about causes of mortality has been added in lines 223-224.

3. p12 line 260-262: It is too early to make such a strong dietary advice based on the sub-analysis of this data set.

*Response:* We appreciate the comment and we deleted the sentence.

4. p6 line 115-117: The reasons of the trial termination should be briefly mentioned.
Response to reviewers

Response: Thank you for the observation. We added a sentence and a reference to explain the reason why the trial was stopped: “The trial was stopped after a median follow-up of 4.8 years due to the benefit of the Mediterranean diets with respect to major cardiovascular events: myocardial infarction, stroke, or death from cardiovascular causes (analysis performed by the Drug and Safety Monitoring Board of the trial) compared to the control group [2],” on lines 131-135.

5. Since the study was based on a dietary intervention study, the diet after the intervention was rather for a short-term and might be different from their original dietary habits. It may be more informative to show the association between baseline diet and mortality.

Response: Changes with dietary intervention are observed even after short periods of time. In the PREDIMED study, after only 3 month of a Mediterranean diet intervention (Estruch et al 2006), we observed significant changes in biochemical parameters such glucose, blood pressure, and HDL-cholesterol concentration. In addition, compared to the control group, a significant reduction in the incidence of major cardiovascular events was observed in both Mediterranean diet groups after a mean follow-up of near 5 years. Thus, this period of time is enough to reach cardiovascular benefits following a Mediterranean diet intervention (Estruch et al, 2013). On the other hand, the cumulative average of polyphenol intake across yearly repeated measurements of diet is considered as the best approach to reduce measurement error in nutritional epidemiology.

Minor Essential Revisions:

There are several English errors.

e.g. neither total polyphenol intake or the different - neither ... nor ...

Response: Thank you for the observation. We fixed the mistake and the full text has been revised by a native English reviewer.

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests: I declare that I have no competing interests.
Response to reviewers

Reviewer: Johanna Dwyer

Discretionary revisions and comments

The use of a moving average intake is interesting (line 151 on).

Thank you.

Some of the findings are counterintuitive, such as the group with high cholesterol levels in blood with low SFA intakes.

Response: This fact is frequently observed in epidemiological studies and it may be explained by a “reverse causality”. That is, subjects with a disease (hypercholesterolemia) reduce SFA intakes following the recommendations of their physicians and dietitians.

What are dihydroflavonols? May want to include other names as well.

Response: Dihydroflavonols are a flavonoids subclass, mainly found in wines.

line 116 : say why trial was terminated (for favoring MedDiet?)

Response: Thank you for the observation. We added a sentence and a reference to explain the reason why the trial was stopped: “The trial was stopped after a median follow-up of 4.8 years due to the benefit of the Mediterranean diets with respect to major cardiovascular events: myocardial infarction, stroke, or death from cardiovascular causes (analysis performed by the Drug and Safety Monitoring Board of the trial) compared to the control group. [2]”, on lines 131-135.

line 91 chronic degenerative disease

Response: Thank you. We add the word “degenerative” in the new line 116.

line 84 with respect to nutrients, the MedDiet is...

Response: Thank you for the observation. We fixed it.

Ref 15 is incomplete

Response: we appreciate the comment. We have completed the reference and reviewed all references.
Response to reviewers

Explain or reference Nelson Aalen estimates; this is an unfamiliar technique for some readers of this journal.

Response: We have added a brief explanation about the Nelson Aalen estimates in the Methods section, lines 194-196.

Major Points

1. How complete was the food table used for polyphenols, and when there were missing values what defaults were used?

Response: The FFQ contained 137 food items and had been validated (Fernandez-Ballart JD et al, 2010). When missing values were found, we used the mean value of the previous and the following year, except for baseline FFQ data. When the baseline FFQ was missing, we excluded the participant.

2. Since the associations found were not linear, why didn't the authors use splines or fit the data to the curvilinear function that seems to exist, instead of tests for trend.

Response: Even though some of the association between polyphenols and the HR did not follow a linear trend, it is a common practice to use $P$-trend to test the linearity of the results. Moreover, each polyphenol group (flavonols, phenolic acids, stilbens, etc.) fit to different functions.

The spline analysis also has limitations, for example it can be highly dependent of the particular data used to generate the spline function and this can limit its generalizability. In addition, the selection of knots also involves some degree of subjectivity. Perhaps this is the reason why only a simple linear trend test is used in most published analysis with more than 2 categories in nutritional epidemiology, regardless of whether a monotonic association is observed or not”.

3. In the interpretation of the final model, how is one to rule out the effects on outcomes of other possible causes, such as PUFA, etc. On pg 11 lines 230-237 was it just associations with lignans and other polyphenols or all that came out to be attenuated?

Response: In order to rule out the effects of intake of other nutrients in the results, in model 3, we additionally adjust for proteins, saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, and cholesterol. However, the association was only attenuated for “other polyphenols” after adjustment for other nutrients. We have rewritten the sentence because there was a mistake (lines 261-263).
Response to reviewers

The explanation of results with model 3 needs expansion, at least for this reviewer. It is difficult to envision how much of the effect was actually "left" after the other constituents of diet were taken into account. What about multicollinearity, such as the effects of alcohol in wines vs stilbenes.

Response: We understand model 3 as an additionally adjusted model. It takes into account the intake of nutrients, which is both correlated with mortality (as diet, in general, affects health and, therefore, mortality) and polyphenol intake. In model 3, we also controlled for alcohol intake as we did in model 2. It is true that stilbenes are mostly found in wines but also in grapes, cocoa, berries, nuts, vinegar, etc.

4. This is really not a observational longitudinal study with an hypothesis as stated in lines 88-93, , and again line 122 and 245, as it is described, but rather a reanalysis of an intervention study, isn’t it? Given that the polyphenols are known to be high in the MedDiet what is unique about showing that they are high?

Response: We agree with the reviewer. This study was “a re-analysis of an intervention study”. Accordingly, we have changed the title of the manuscript and included this statement in the Material and Methods. However, in this sense, it is important to realize that in the PREDIMED trial, the three groups (two with Mediterranean diet plus olive oil or nuts, and the control group) were having similar amount of polyphenols. There were no statistical differences among the three groups, because the control group also followed-up a diet rich in polyphenols. In fact, there were not differences among the three groups on the consumption of fruit and vegetables, the main sources of dietary polyphenols in the trial.

6. With so many covariates, was there a danger of overadjustment?

Response: We agree with reviewer that the main danger in this type of statistical analysis is “over-adjustment”, but this kind of adjustments is the common practice in these analyses. For this reason, we presented the results of 3 different models with different levels of adjustment. We included variables that are associated with the exposure variable and the outcome. However, we did not include in the model variables that did not change the HR by 10% or more.

5. The discussion is too long and overly speculative and needs to be edited down

Response: Thank you for the comment. We modified the discussion according to this suggestion.

Quality of written English: Needs some language corrections before being published
Response to reviewers

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:** I received a one time fee and travel expenses from Ocean Spray Cranberries for attending a meeting in 2013.
Editorial comments:

I also don't understand using a linear test for trend for what seems a curvilinear association. And why no nonferrous or other corrections for all the multiple testing?

Response: Even though some of the association between polyphenols and the HR did not follow a linear trend, it is a common practice to use $P$-trend to test the linearity of the results. Moreover, each polyphenol group (flavonols, phenolic acids, stilbens, etc.) fit to different functions.

The spline analysis also has limitations, for example it can be highly dependent of the particular data used to generate the spline function and this can limit its generalizability. In addition, the selection of knots also involves some degree of subjectivity. Perhaps this is the reason why only a simple linear trend test is used in most published analysis with more than 2 categories in nutritional epidemiology, regardless of whether a monotonic association is observed or not".

Additionally, there are a small number of editorial revisions that should be made at this stage, which I have listed below:

1. Please provide the names of the ethical committees that gave approval for your study. If the list is long you may wish to include it as an additional file and refer to the file in the main text.

The study protocol was approved by the Institutional Review Board of all the centers that participated in the recruitment and assessment of the participants. These centers were the following: Hospital Clinic of Barcelona (coordinating centre), Universities of Barcelona, Valencia, Rovira-Virgili (Reus), Malaga and Las Palmas, Municipal Institute for Medical Research, Primary Care Division of Barcelona and Seville, Institute of Research in Health Sciences (IUNICS) at Palma de Mallorca, Hospital Txangorritxu of Vitoria, and University Hospital of Bellvitge (lines 135-141).

2. Authors' contributions: Please ensure all authors are mentioned here. Some authors appear to be missing. More information about authors' contributions can be found here:

http://www.biomedcentral.com/bmcmed/authors/instructions/researcharticle#formatting-ontributions

Response: Thank you for the comment. We have added the author who was missing.
Response to reviewers

Please also ensure that your revised manuscript conforms to the journal style (http://www.biomedcentral.com/info/ifora/medicine_journals). It is important that your files are correctly formatted.

Response: We have revised the manuscript and modified according to the Journal style.