

Author's response to reviews

Title: Plasma micronutrient status is improved after a 3-month dietary intervention with 5 daily portions of fruits and vegetables: implications for optimal antioxidant levels

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Author's response to reviews: see over

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Re: MS: 1660290793208928

“Plasma micronutrient status is improved after a 3-month dietary intervention with 5 daily portions of fruits and vegetables: implications for optimal antioxidant levels”

Maria Cristina Polidori, Juan Carlos Carrillo, Pablo E Verde, Helmut Sies, Johannes Siegrist and Wilhelm Stahl

Dear Editorial Team,

please find attached a revised version of our above cited article. The point-by-point responses to the reviewer are enclosed hereafter.

With best regards,

M. Cristina Polidori on behalf of all Coauthors

Reviewer 1.

We thank this reviewer for his appreciation and comments.

1. Abstract: contrary to what is stated, according to your table 2, there was NO statistically significant difference between plasma levels of zeaxanthin, β -cryptoxanthin, retinol, and α -tocopherol at T0 and T3 !?!

We apologise for the misunderstanding. In the abstract, we state ‘especially the intake of fruits was significantly improved after 3 months of intervention, and plasma levels of lutein, zeaxanthin, β -cryptoxanthin, lycopene, α - and β -carotene, retinol, α -tocopherol, vitamin C and vitamin B6 were increased’. While the intake of fruits was significantly improved, mean plasma levels of micronutrients were increased. We did not specify, due to space problems, for which micronutrients significance was achieved.

2. Abstract: given that the majority of eligible participants were women (86%), you can not generalize your findings to “a health-conscious population”, thus to both men and women.

We added the 86% value in the abstract so that the readers know that mainly women participated.

3. Methods: strictly spoken, participants were not randomly assigned to the

targeted dietary counseling of interest or, for instance, to a more general healthy lifestyle advice (placebo), but all participants were subjected to a single intervention strategy. Thus one can not be sure that the observed changes in blood levels of markers of interest were due to the dietary intervention of interest as a control group was lacking.

We added a clarifying sentence in the discussion.

4. Methods: although 112 subjects were recruited in the study and were said to have complete datasets, numbers in each of the 3 cells in table 1 add up to 100 only!?! Does this mean that 12 subjects were lost to follow-up? What were the reasons for lost to follow-up? How many of these were male and how many female?

Values displayed in Table 1 are expressed in percentage.

5. Methods: the meaning of the dynamic migration model for assessing compliance is unknown to me and needs further clarification.

We could not include more explanation on the dynamic migration model for space reasons. We add hereafter a clarifying paragraph for the Editors and the Reviewers.

The nutritional behavior was evaluated using the nutritional index developed by Winkler and Döring (ref 2 of the list). The overall Index has a total possible score ranging from 0 to 32. The higher the index score, the better the diet conformed to the dietary guidelines recommendations given by the German Nutrition Society (Deutsche Gesellschaft für Ernährung, DGE, www.dge.de). The resulting score was classified according to the three categories defined by Winkler and Döring: A = optimal (score greater than 16), B = normal (score between 14 to 16 points) and C = poor nutritional behaviour (score less than 13 points). Questionnaires were collected at the end of each of the four sessions and at home between two consecutive sessions. At the end of the study, therefore, seven questionnaires were available for each participant to assess intake of fruits and vegetables and in order to monitor nutritional changes over time more reliably. The dynamic migration model was used to assess compliance, as it measures the probability to move from a poor nutritional class according to the index developed by Winkler and Döring to a better one. The migration behavior model allows to confirm the compliance to the nutritional intervention when the chance to migrate to upper class categories is higher than to migrate to the lower ones during the intervention time. Significance was accepted if the null hypothesis was rejected at the $p < 0.05$ level. According to the classification of nutritional behavior, the distribution at baseline comprised 67% of the subjects with optimal nutrition (A), 8% with normal nutrition (B) and 25% with poor nutrition (C). For fresh vegetables the distribution was: 27% A, 46% B and 27% C, for boiled vegetables 12% A, 57% B and 31% C, for fresh fruits 69% A, 25% B and 6% C. On the basis of the migration behavior model, a good compliance to the intervention ($p < 0.01$) as well as a substantial tendency of the study participants to remain in the nutritional class to which they belonged for the entire duration of the study were observed concerning the intake of fresh and boiled vegetables. As far as the intake of fresh fruits is concerned, not only it improved ($p < 0.01$), but also a significant difference between study periods 1 and 2 was achieved ($p < 0.01$).

6. Statistical analyses: should contain a paragraph on exact definition of confounders, eg. age in 1-year categories and exact definition of smoking status (see also point 8).

We added one sentence about confounders. Smokers did not change their smoking habit during the whole course of the intervention study. As the statistical analysis was rigorously performed correcting for smoking habit together with other variables, the observed changes in micronutrients are not related to smoke.

7. Results: fruit intake has improved considerably, while #-Cryptoxanthin is a good marker of fruits, why then are concentrations of this carotenoid not increased, but even show a suggestive decline in table 2??

This result might be due to the fact that the study subjects improved the intake of fruits other or mainly other than citrus fruits, particularly rich in β -cryptoxanthin.

8. Results: the footnote of table 2 reads that concentration differences over calendar time were corrected for age, gender and smoking status. Wondered how well smoking was taken into account since improvement in smoking behaviour may partially explain the observed changes in micronutrient levels. For instance, did you assess changes in smoking intensity? And did the 12% smokers change their smoking habits, eg. did they quit or change their number of cigarettes? Thus, you need to better define your variable smoking status and provide evidence that smoking does not underly the observed changes in carotenoid levels.

Please see point # 6.

- Minor Essential Revisions

1. General: the lay out of this brief scientific report lacks the customary division into Introduction, Material and Methods, Results and Discussion.

This is a short report article and as such it does not have to be divided into the usual original report sections.

Reviewer 2.

We thank this reviewer for his appreciation and comments.

Reviewer's report:

This manuscript shows that an increase in the intake of fruits and vegetables for three months leads to enhanced levels of certain antioxidants (vitamin C, lycopene, lutein, and carotenes), but does not affect markers of oxidative stress. The lack of effect on biomarkers of oxidative stress seems to be due to the high consumption of fruits and vegetables that exhibited the recruited population at the beginning of the study. This study helps to understand the effects of increasing fruit and vegetable intake and the limits of its effect on systemic oxidative stress. This work is of interest in the field, nevertheless this reviewer has the following comments as discretionary revisions:

- Regarding MDA measurement in plasma, it is recommended the use of

EDTA-treated blood instead of heparinized blood to avoid an artificial increase in lipid peroxidation during sample processing. Nevertheless, the plasma MDA levels reported in the present manuscript are certainly low and consequently sample preparation seems adequate.

This is an important point, however we did use exclusively non-hemolysed plasma samples and therefore we did not need to complexate free iron.

- The lack of effect of increasing fruits and vegetables intake on MDA levels might be due to the absence of changes in vitamin E levels, and this should be discussed. Indeed, Table 2 shows that alpha-tocopherol levels do not change significantly after improving the intake of fruits and vegetables.

We agree with this reviewer and added a sentence in the discussion.

- The lack of significant effect of increasing antioxidants on biomarkers of oxidative stress might be due to the high levels of antioxidants already present at the beginning of the study, but it should be considered in the discussion the hypothesis that in the short term it may be inefficient to increase antioxidant levels in a healthy population not subjected to any kind of pathological-related oxidative stress. However, this approach may be useful in the long term in order to prevent or diminish age-related chronic oxidative stress.

We agree with this reviewer and modified the relative sentence in the discussion.

- There are some relevant reviews on this issue, such as those by Hamer and Chida (J. Hypertension 2007), Voutilainen et al. (Am. J. Clin. Nutr. 2006), Neuhouser (Nutr. Cancer 2004), and Martin et al. (J Nutr. Health Aging, 2002), which should be cited.

We thank this reviewer and are aware of pieces of literature that we unfortunately cannot cite for space reasons.