Author's response to reviews

Title: Sparing effects of selenium and ascorbic acid on vitamin C and E in guinea pig tissues

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Author's response to reviews: see over
Re: MS: 1319438891110613 - Sparing effects of selenium and ascorbic acid on vitamin C and E in guinea pig tissues

Dear Nehme Gebrayel,

We have resubmitted a revised version of the manuscript entitled “Sparing effects of selenium and ascorbic acid on vitamin C and E in guinea pig tissues”. We would like to thank both reviewers for their comments and suggestions. Reviewer 1 provided a very positive review indicating that “The study is well designed and conducted; the manuscript is well prepared and easy to follow and interpretations and conclusions are fair” and only had a few suggestions for discretionary revision. Reviewer 2 also provided a positive review indicating that “The question posed is very important” and “could impact human nutrition in important ways”. In preparing this version of the manuscript we have addressed all comments provided by the reviewers. A point-by-point response to the reviewers’ concerns is outlined below.

Reviewer 1

Discretionary Revisions

1) Methods: The authors report that AA was given via oral dosage. This is not clear. Was a gastric tube used for this?

Response:

Guinea pigs were orally dosed by gavage. Dosing was performed with a syringe (without the needle) that was inserted into the guinea pig’s mouth. The AA solution was then dispensed into the cheek area. Swallowing of the entire AA dose was ensured. The Methods section has been revised to include that the oral dosing was by “gavage” (page 5, line 7).

2) Results: AT concentrations: The authors state (page 10, bottom and page 11, top) that there was no difference in AT concentrations at week 5 between the four groups. This is correct with respect to statistical evaluation of these data. A view on Table 4, however, shows that concentration in most of the tissues was clearly lower in SeD/MC than in the other groups. Undoubtedly, the reason for the lack of statistical significance is that SEMs were relatively high and n was relatively small. I would recommend to point out that AT concentrations in the SeD group were lower than those in the SeM and SeN groups although there were no significant differences. Maybe there was a tendency (P<0.10 or 0.15)? Similar is true for liver and plasma lipid peroxide levels. There is a clear trend towards a reduction from SeD via SeM to SeN.

Response:
Reviewer 2 suggested that we refrain from characterizing results that approach significance [Minor Essential Revisions (1)]. The manuscript has been revised to exclude characterization of results that are not significant at $P < 0.05$. We also feel that this will not detract from the main findings/conclusions of the paper.

Reviewer 2

Major Compulsory Revisions

1) The 5 and 12 week groups have been combined for statistical purposes. That does not seem appropriate to this reviewer because not only were they at different times but the time of sampling was 24 h after AA dosing in one case and 48 h after it in the other. Thus, there were 2 differences between the groups and yet they were combined. Clear justification for this is needed.

Response:

The reason for combining these data was to increase the power of the statistical comparisons. Even though the data are from guinea pigs of different ages and times post AA dosing, combining these data is considered by our biostatisticians to be statistically acceptable because the response (i.e. vitamin C concentrations) to changes in dietary selenium or ascorbic acid was similar for week 5, 48 hrs and week 12, 24 hrs guinea pigs. Similarity of the response was checked by ANOVA prior to combining the data. We have revised the Methods section to emphasize this point (page 8, lines 1-6).

Data for guinea pigs killed at these specific times were combined because a discernable decreasing trend in vitamin C concentrations in most tissues was observed for these guinea pigs, but not for guinea pigs killed at the other times (i.e. week 5, 24 hrs and week 12, 48 hrs). The reason for the observed Se effect on vitamin C at different times post AA dosing for week 5 and 12 guinea pigs is not known, but may be due to differences in the metabolism of the dosed AA between younger (week 5) and older (week 12) guinea pigs. The Discussion includes this possible explanation for the difference (page 13, lines 17-21 and page 14 lines 1-17). Further, although combining these data was performed post hoc, sparing effects of selenium and AA on vitamin C were observed across most tissues indicating that the observed effects are real.

2) This work represents a single experiment. The results in Figure 1 are at variance with work in the literature. These results show DHAA as most of the vit C in many tissues. The authors claim only that their data must be correct without supporting it experimentally. At least they could carry some AA and DHAA through their procedures and show it does not change to the other form. Even better would be to confirm the experiment.

Response:
It should be noted that there is limited data in the literature describing vitamin C in guinea pig tissues and that vitamin C metabolism is distinctly different in guinea pigs compared to rodents which have the ability to synthesise vitamin C. The large proportion of vitamin C detected as DHAA in tissues from guinea pigs in this study is in agreement with two earlier studies measuring vitamin C in guinea pig tissues. A study by Hidiroglou et al [reference 40] reported comparably high DHAA/AA ratios in tissues using this same method for vitamin C determination. Further, a study by Martensson et al [reference 41] also reported large DHAA/AA ratios in liver and kidney of guinea pigs fed an ascorbate-deficient diet using different methodology to measure vitamin C. Importantly, tissue vitamin C concentrations of guinea pigs in the study by Hidiroglou et al and in this study are comparable to the vitamin C concentrations in tissues from guinea pigs fed an ascorbate-deficient diet in the study by Martensson et al and are considerably lower than the concentrations reported by Martensson et al for guinea pigs fed higher levels of vitamin C. Given these data, we postulate in this paper that a low vitamin C diet leading to low vitamin C concentrations in tissues promotes an increase in DHAA/AA ratios in guinea pig tissues. This concept is supported by data presented in the study by Martensson et al showing that most of the vitamin C was detected as AA in tissues of guinea pigs fed a higher vitamin C diet. An explanation for the large DHAA/AA ratios reported has been included in the Discussion section (page 16, lines 7-21).

Two papers have been published that describe the method used in this paper for the determination of vitamin C [references 25 and 27]. As part of the method development, the experiment suggested by the reviewer has already been performed. AA and DHAA standards spiked into tissue homogenates can be completely recovered using this method. Time course experiments showed that AA and DHAA remain stable in a tissue matrix over 90 minutes indicating that there is little conversion of AA to DHAA in the sample. In addition, AA determinations were compared to AA determinations using a separate method [reference 28] and were shown to be comparable. To prevent oxidation of vitamin C in tissues prior to analysis, excised tissues were immediately frozen in liquid nitrogen and plasma and tissue homogenates were treated with metaphosphoric acid. The Methods section has been revised to include a more detailed description of the methodology and control experiments that have been performed (page 6, lines 3-17).

3) Some of the guinea pigs fed low selenium and minimal C became paralyzed and some died. Why? This is potentially very important in understanding the function of these nutrients. Why did some die and some survive? What were animal weights? More about this needs to be put into this manuscript.

Response:

We agree that the paralysis and death observed in guinea pigs fed a combined selenium-deficient and marginal C diet is an important outcome measure of this study and should be investigated further. However, this is not the main focus of this manuscript. We have, however, expanded the Discussion section to include an explanation of these results and
how they compared with similar studies describing the effects of deficiencies in these nutrients in guinea pigs (page 13, lines 10-16).

We also agree that in dietary animal studies body weights can provide important information on the general health of the animals and are often useful in interpreting data. However, for this particular paper we feel that including guinea pig body weights will be of little value to the interpretation of the data and will unnecessarily increase the length of the manuscript. Only a small decrease in body weight (approximately 10% decrease at week 12) was observed for Se-D/MC guinea pigs compared to guinea pigs in the other diet groups.

Minor Essential Revisions

1) The manuscript needs to be more concise. A great deal of space is taken up by presentation of results that “approach” significance. The authors should refrain from such characterization. Those results are “not” significant.

Response:

References in the text to data that approach significance have been removed.

2) Too much space is devoted to description of results at the expense of the meaning of those results. For example, on Page 15 it is asserted that a sparing effect of AA on Se-GSH-Px activity suggests that AA intake influences Se status. Se-GSH-Px activity can be affected by other metabolic and stress factors than just Se status. It seems more likely that such effects would be active here rather than an effect on Se status.

Response:

We have included in the Discussion the possibility that the decrease in Se-GSH-Px activity observed with AA restriction may have resulted due to an effect on a biochemical process that influences Se-GSH-Px activity (page 17, lines 5-7).

In closing, we hope that you will agree that we have addressed all the reviewers’ concerns appropriately and find the manuscript acceptable for publication in Nutrition Journal. We thank you for your time in considering our manuscript.

Sincerely,

Jesse Bertinato, Ph.D.
Research Scientist