Author's response to reviews

Title: Copper Chaperone for Cu/Zn Superoxide Dismutase is a sensitive biomarker of mild copper deficiency induced by moderately high intakes of zinc

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Version: 2 Date: 10 November 2005

Author's response to reviews:

November 10, 2005

Dear Hiromichi Kumagai,

We have submitted a revised version of the manuscript entitled "Copper Chaperone for Cu/Zn Superoxide Dismutase is a sensitive biomarker of mild copper deficiency induced by moderately high intakes of zinc". In preparing this version of the manuscript we have addressed all comments provided by the reviewer and have made changes to the text and tables.

1. We would like to begin by thanking the reviewer for his/her comments and helpful suggestions. Overall, the reviewer provided a positive review indicating that "this study is interesting" and "the manuscript is well written". The major concern, however, was the lack of response of WBC CCS to elevated zinc intake given the sensitivity of erythrocyte CCS. In the original version of the manuscript we provided an explanation as to why we believe statistically significant differences were not found for WBC CCS and have included this explanation in the revised version (Page 16, lines 16-21 and Page 17, line 1). Briefly, we believe the reason is the over sampling of rats with better copper status (i.e. the majority were characterised as non-responders for decreased plasma copper). However, we preformed the analyses suggested by the reviewer and compared CCS expression in liver, erythrocytes and WBCs between responders and non-responders for plasma copper and have included these data in a separate table (Table 4). These data show that WBC CCS is significantly higher in responders compared to non-responders fed normal Zn (Zn-30), indicating that WBC CCS is responsive to Cu deficiency induced by moderately high intakes of zinc. We have described these data in the Results section (Page 13, lines 12-15) and provided an interpretation of the data in the Discussion (Page 15, lines 11-16; Page 17, lines 3-6). Although RBC CCS is shown to be a sensitive measure of mild Cu deficiency, WBC CCS may prove to have value in detecting early reductions in Cu status that might not be detectable in erythrocytes given the slow turnover of erythrocytes. We feel that this statement should be included in the Discussion (Page 17, lines 6-7).

We have rephrased the sentence on Page 17, line 2 in the original version of the manuscript. In this statement we want to report on an observation that we have made that the basal expression of CCS in WBCs is much higher than in other tissues such as liver, heart and erythrocytes. Since this is the first report describing CCS expression in WBCs, this information may be of interest to researchers in the field (Page 17, lines 7-9).

2. The reviewer noted that the horizontal line in the Zn-60 group in Figure 1 is not the mean of the responders shown in Table 3. The reason for this discrepancy is that the plasma Cu and Cp data presented in Figure 1 and Table 3 have been analysed using two different statistical approaches. The maximum likelihood approach was used in Figure 1 to test for differences in the response means and the non-response mean. In Table 3, a cut-off value of 2 standard deviations of the mean of the control group (Zn-30 rats) was used to identify individual rats as responders and non-responders. The Methods section (Statistical Analyses) has been revised to clarify this point (Page 10, lines 6-19).
The maximum likelihood approach was used in Figure 1 to analyse differences between the means of the responders and the non-response mean. However, the maximum likelihood approach does not classify individual observations as responders and non-responders but finds the bimodal distribution that best fits the data. In some cases there is sufficient separation between responders and non-responders to classify them with a low probability of miss-classification, but when there is little separation between responders and non-responders (like in the Zn-60 group) then the probability of miss-classification can be quite high. Therefore, to characterise rats as responders and non-responders a cut-off value of 2 standard deviations from the mean of the control group (Zn-30) was used (Table 3). Although the use of a cut-off to define responders provides a means of summarizing the data, it too suffers from the same problem of miss-classification, but does provide summary information that is easily understood and has been used previously when comparing similar data (ref. 9 in the manuscript). However, the maximum likelihood estimates are the valid estimates of the response and non-response means and should also be retained in the discussion.

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

Sincerely,

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