

Author's response to reviews

Title: Soy protein supplementation does not cause lymphocytopenia in postmenopausal women

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Version: 2 **Date:** 9 March 2006

Author's response to reviews: see over

March 9, 2006

Hikomichi Kumagai, MD
Deputy-Editor-in-Chief
Nutrition Journal

Dear Dr. Kumagai:

Thank you for the feedback provided in response to manuscript (MS: 8789798158544577) entitled "Soy protein supplementation does not cause lymphocytopenia in postmenopausal women". We have revised the manuscript in accordance with the reviewers' comments and recommendations. The reviewers' comments were very helpful in enhancing the quality of this paper. We have addressed all of the reviewers' comments by making revisions to the manuscript.

Attached, please find our responses to the specific comments made by the reviewers. If you have any questions, please call me at (405) 744-4437 or e-mail me at arjmand@okstate.edu.

Sincerely,

Bahram H. Arjmandi, PhD, RD, LD
Regents Professor of Nutritional Sciences and Gerontology

Manuscript ID: 9039340675725514

Title: One year soy protein supplementation has positive effects on bone formation markers but not bone density in postmenopausal women

Authors: Arjmandi BH, Lucas EA, Khalil DA, Smith BJ, Devareddy L, McDonald J, Arquitt AB, Payton ME, Mason C

Response to the Reviewer's Comments

Manuscript No: 8789798158544577

Corresponding Author: Arjmandi BH.

We thank the reviewers for their enthusiasm regarding our manuscript. Their suggestions were very helpful in enhancing the quality of our paper. We have carefully considered the reviewers' comments in revising the manuscript.

Reviewer

1:

Comment 1. "Statistical considerations: The data appear to lend itself to a 1x2 analysis of covariance (ANCOVA), using pretreatment values as a covariate."

Response 1. We agree with the reviewer that ANCOVA would be an alternative to the approach we took. We chose to have time as a factor with levels at baseline and final. Another way to analyze the data is to use baseline values as a covariate or express the various measured parameters as a percentage change from baseline. Hence, the authors suggest that the approach taken should be considered quite appropriate.

Comment 2. "General comment related to the background and discussion: Bone/Osteoporosis: The descriptions regarding soy effects on the skeleton are unbalanced. The current evidence suggests that dietary soy protein and/or isoflavones (phytoestrogens) have only modest (if any) effects on the skeleton, and do not significantly prevent bone loss in post-menopausal women. A prior publication from this study found no effect of soy supplementation on bone mass in these same subjects. Given these weak effects on bone, it is not appropriate to view soy and/or isoflavones as a substitute for accepted therapies such as estrogen, bisphosphonates, PTH analogues, etc."

Response 2. We agree with the reviewer about the modest effect of soy and/or isoflavones in preventing bone loss in postmenopausal women. We have revised the introduction section to reflect the different findings on the effect of soy on bone (page 3, lines 2-13). (page 3, lines 2-13).

Comment 3. “The "change" from baseline in basophil counts in both groups might be an instrument calibration problem. Automated basophil counts have a high coefficient of variation. What were the intra- and inter- assay coefficients of variation for the CBC parameters?”

Response 3. We thank the reviewer for bringing the issue of variance in automated basophile count determinations to our attention. We have reviewed the inter- and intra assay CVs for this parameter in our laboratory and have found that the differences obtained due to treatment in this study are within the range of CVs for this parameter. As such, the differences obtained due to treatment are not considered significant. We have revised the Abstract, Results, and Discussion sections of the manuscript accordingly. We have included the CVs for the CBC parameters in the Methods section of the manuscript (page 5, lines 17-19).

Comment 4. “Needs some language corrections before being published”

Response 4. We thank the reviewer for bringing this to our attention. The manuscript has been read and corrections have been made.

Reviewer 2:

Comment 1. “How do you subjectively measure the compliance of the participants? Is there any blood drawn showing the level of genistein?”

Response 1. The authors agree that measurement of the level of genistein in blood would be the best way to ensure compliance by the study participants but this was not assessed in this study. From our experience, we have seen that just before blood draw, subject expect some kind of compliance monitoring and therefore they take the supplement the day prior to coming for blood draw. However, compliance was monitored by several means: 1) Every subject were provided with a calendar, for daily recording of their intakes of the treatment regimen, which were given with each package of supplies on a monthly basis. 2) Each subject met every month with the investigators for monitoring weight and provision of study supplies. At this monthly meeting, each subject received her supplies for the next month along with a calendar. 3) Unused packets of treatment regimen were returned and counted at these monthly site visits. 4) Each study participants were contacted via telephone on a random date between the study site visits to encourage compliance. 5) In addition, each subject received frequent postcards with reminders to consume the study regimen.

Comment 2. “Blood collection was only performed twice at the beginning and end of the study, which can hardly show the trend and curve of the hematological changes of the participants.”

Response 2. Understandably, it is difficult to conduct covariance or regression with two time points. However, since the present study was part of a larger study with different

objectives, we therefore could only utilize the available two blood samples from those time points. The authors are certain that the reviewer agree that this would not be considered a limitation in this study although multiple blood draws would have been preferable.

Comment 3. “There was high drop out rate (25/87), which will definitely affect the power of the study.”

Response 3. True, high drop out rate has reduced the power of the study. However, this has not precluded us from observing differences in some of the assessed hematological parameters. Nonetheless, the lack of effect of soy supplementation on lymphocyte counts cannot be attributed to the smaller than anticipated sample size because not even a slight trend towards an effect could be detected from the data.

Comment 4. “Need more detailed description of inclusion and exclusion criteria of the study.”

Response 4. We have revised the Methods section of the manuscript to include a more detailed inclusion and exclusion criteria for the study participants (page 4, lines 16-19).

Comment 5. “More detailed description of the control protein.”

Response 5. The control protein foods consisted of a milk protein-based drink mix, a wheat protein-based cereal, and a peanut protein-based snack bar which were all devoid of isoflavones. This is now included in the Methods section of the manuscript (page 5, lines 2-5).

Comment 6. “In "Conclusion", it should be stated that "60 mg soy isoflavone does not.”

Response 6. We thank the reviewer for bringing this to our attention. We have revised the conclusion to state that soy protein containing 60 mg isoflavones does not significantly alter hematological parameters in postmenopausal women (page 10, lines 2-3).