Author’s response to reviews

Title: Salivary Secretory Immunoglobulin A secretion increases after 4-weeks ingestion of chlorella-derived multicomponent supplement in humans: a randomized cross over study

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Author’s response to reviews: see over
Responses to the Nutritional Journal Editorial Team’s and the Reviewer’s Comments (Re: MS: 8527035085181997)

Authors: We appreciate your constructive comments and suggestions. We have considered all the comments carefully and revised our manuscript accordingly. Our responses to each comment are as follows.

The Nutritional Journal Editorial Team:

The authors need to address all the major critiques from Reviewer 2 for this paper to make it through.

Authors: We completely agreed with the Editorial Team and addressed all the major critiques from Reviewer 2. Please see the responses to the Reviewer 2 and the revised manuscript.

Reviewer 1:

General Comments
A recurring motif that runs through the entire length of the paper is the lack of adequate discussion, and the writing is quite poor.

Authors: According to the reviewer’s comments, the ‘Discussion’ section was revised. Also, we revised our manuscript from the view point of English syntax. Please see the following responses and the revised manuscript.

My major concern is how much impact does the chlorella have on mucosal immune function. Indeed, the authors showed significant increase in salivary SIgA after 4-weeks chlorella ingestion, however absolute levels of SIgA look not very different form that of placebo group (And the SE looks very wide). I do not think this small difference makes any biological effects in general.

Authors: As correctly pointed out by the reviewer, the inter-group difference in salivary SIgA level after the ingestions is not large and the SE is not small. Also, we recognize a lack of data describing the clinical significance is a study limitation. However, since SIgA plays a crucial role in mucosal immune function as the first line of defense (Lamm ME et al., APMIS 1995), it is possible the small change observed in this study made biological effects. Indeed, Yamauchi et al. (Int J Sports Med 2011) reported the expression of Epstein-Barr virus-DNA in saliva and the increase in a number of upper respiratory symptoms occurred on the following day of approximately 23 percent reduction in salivary SIgA secretion rate, although this reduction was smaller than the increase by the chlorella supplementation in our study (41 %). Again, the salivary SIgA secretion rate after the chlorella ingestion was 37 percent greater than that after the placebo supplementation. Therefore, we consider the increase in salivary SIgA secretion rate in the chlorella trial was clinically significant.
We added this issue in the revised manuscript (Page 8, Lines 10-20).

Specific comments:
There are substantial amount of grammatical errors in the manuscript.

Authors: According to your request, we revised our manuscript from the view point of English syntax. Please see the revised manuscript.

In general, SIgA values are not stable in the morning, especially before breakfast. The reviewer suspects the authors found wide-variation of the value. If this is not the case, how do the author control the subjects or condition of sample collection. There is a lack of the descriptions of details in the method section.

Authors: We appreciate the reviewer’s insightful comments. This manuscript shows the data of all subjects who participated both in the placebo and chlorella trials. The variations we found were identical to those demonstrated in Figures 1. According to the Reviewer’s advice, we added the descriptions of details in the ‘Method’ section (Page 4, Lines 16-17; Page 5, Lines 12-15).

Do you have any data showing SIgA values are stable in the morning?

Authors: We obtained saliva in the morning to equalize the condition of subjects among four sampling points (placebo or chlorella trials x before and after ingestions). However, as correctly pointed out by the Reviewer, salivary SIgA level is not stable in the morning especially during 10 min after awakening (Hucklebridge F et al., Int J Psychophysiol 1998). Although the saliva collection in this study was performed at least 1 hour after awakening, we can not rule out the effects of diurnal cycle in salivary SIgA secretion. This issue was added as a study limitation (Page 8, Lines 20-25).

The author should add adequate references to the second paragraph, line 3.

Authors: In agreement with the reviewer, the authors added the references (Page 3, Lines 19-20).

The reviewer does not think the 3rd paragraph in the discussion helps to understand the results of the study because the author never showed any data regarding with.

Authors: According to the reviewer’s advice, this paragraph has been deleted.
Generally speaking, lack of protein leads decreased immune functions. However, there are very few studies showing normal nutrition with supplements increase immune function. If you would prove this, the authors have to show evidences that subjects took enough energy intake (especially protein intake).

Authors: As the reviewer correctly pointed out, this paper needs the evidence that subjects took enough energy and protein. Although we could not investigate their eating habits and perform any blood chemical analysis, it is possible to speculate, from lean body weight, body fat, and body mass index, their intakes of energy and protein were not insufficient. This issue was included in the revised manuscript (Page 6, Lines 8-12; Page 8, Lines 3-6; Table 2).

Reviewer 2:
The authors themselves state in the discussion that other components in the supplement influence IgA levels, and therefore the whole premise that it is the chlorella that is the reason for the apparent beneficial effect (as outlined in the introduction) can simply not be confirmed. The authors themselves say in the discussion that this is the case, and also (rather worryingly) say that the participants themselves may have been of unequal nutritional status, i.e. the protein or vitamin content of the tablets may have acted to correct some underlying deficiency and hence the positive outcome (so why not check this beforehand?). As the authors were clearly aware of these confounding influences, it would have been prudent to use a placebo that differed only to the multi-component supplement in the chlorella content (incidentally there are no details of the placebo in the paper). The study design is not well controlled and this is of major concern and renders the findings somewhat meaningless with regard to the initial research question posed.

Authors: The reviewer's point regarding multi component in the chlorella tablets is well appreciated. Chlorella, a unicellular green alga, is a dietary-supplement material which contains various nutrients. It is an important advantage as the material although we do not negate the existence of this alga’s distinctive ingredient. In the previous studies investigating the immunoenhancing effects of chlorella-derived supplement, the placebo capsule was made from microcrystalline cellulose (Halperin SA et al., CMAJ 2003) or no restrictions were imposed on the control group (Nakano S et al, J Med Food 2007). According to these previous studies, we attempted to make a large difference in nutritive value between the placebo and chlorella tablets. As pointed out by the reviewer, the ‘Introduction’ section was not enough to explain the concept of our study. We revised this section to clearly describe the purpose of this study (Page 3, Lines 15-18; Page 4, Lines 4-9).

We completely agreed with the reviewer that deficit in data regarding nutritional status in the subjects is a study limitation although we can partially speculate their nutritional status based on lean body weight, body fat percentage, and body mass index. We added these values in Table 2 and a description in the ‘Discussion’ section (Page 6, Lines 8-12; Page 7, Line 29-Page 8, Line 10; Table 2). However, this is the first study to evaluate the effects of chlorella-derived dietary
supplement on mucosal immune function in humans. Though mechanisms responsible for chlorella intake-related increase in salivary SIgA secretion were unclear, we consider that this study is valid as an initial step to elucidate the immunoenhancing effects of chlorella ingestion. This issue was also added in the revised manuscript (Page 7, Lines 2-3).

Following the reviewer’s suggestion, more explanation for placebo tablets was added (Page 5, Lines 5-8).

Authors: We would like to express our appreciation to the Nutritional Journal Editorial Team and the reviewers for taking the time to make suggestions for improving our manuscript.

Additional References in the Revised Manuscript: