Author’s response to reviews

Title: B vitamins related to homocysteine metabolism in adults celiac disease patients: a cross-sectional study

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Version: 3 Date: 16 September 2015

Author’s response to reviews: see over
Dear Dr. Hiromichi Kumagai
The Nutrition Journal Editorial Team

Please find attached the revised version of our manuscript (ID 7871373471632831), entitled "B vitamins related to homocysteine metabolism in adults celiac disease patients: a cross-sectional study".

We thank the reviewers for their valuable contributions. We considered the pertinent suggestions and report that all recommendations were taken into account in drafting this new version of the manuscript.

Below we have included answers to the reviewers. Please feel free to contact me for any further questions.

On behalf of the authors.
Sincerely,

Flávia Xavier Valente

Reviewer(s)' Comments to Author:

Referee 1:
1) In the section “Introduction” I would suggest to add MTHFR and MTRR gene mutations prevalence in the homozygous and heterozygous genotype, in general population. These is important to understand that homocysteine metabolism doesn’t only depend by serum concentration of B vitamins. Its prevalence, moreover, does not appear to be higher among celiac patients (Fabbri E, Rustignoli L, Muscari A, Puddu GM, Guarino M, Rinaldi R, Minguzzi E, Caio G, Zoli M, Volta U. Recurrent ischemic strokes in a young celiac woman with MTHFR gene mutation. World J Gastroenterol. 2012;14;18:3472-6).

Author Answer: We add a discussion about MTHFR and MTRR gene mutations, showing its prevalence in general and celiac disease population. However, we decided to include this information on the discussion section, since those mutations were not assessed in this paper.
2) I would suggest to study MTHFR and MTRR enzyme activity in all enrolled patients, specially in two patients presented hyperhomocysteinemia.

Author Answer: During the study planning, it was not aimed to study MTHFR and MTRR enzyme activity. For that reason, we ran out of biological samples, collected during the study, when we performed the analysis initially programmed. This fact prevents us to complement the study with data from enzyme activity. Added to this, we observed low prevalence of hyperhomocysteinemia in celiac disease patients (5%, n=1), so absence of such analysis does not invalidate the study.

Reviewer(s)' Comments to Author:
Referee 2:
1. Why 39 controls were selected (20 patients enrolled)?

Author Answer: Our statistical consultant suggested increasing the sample size by pairing the groups in the proportion of 1 celiac patient to 2 health controls, since many of the potential celiac patients had to be excluded due to the strict inclusion criteria adopted at the study (Figure 1). Therefore, we obtained a study power of 98%, which indicates that our results show a 98% chance of identifying actual differences between groups, even with few celiac patients.

2. The unbalanced proportion of males and females could have some consequences and perhaps a balance of sexes should be more appropriate.

Author Answer: Currently, the literature shows that a characteristic of celiac disease is to affect more women than men [1,2]. In addition, the ratio of celiac women and men observed in our study reflects the prevalence described both national study [3] and international ones [4-8]. Therefore, we believe that a balance between genders would not be an appropriate approach since it would not reflect actual characteristic of the studied disease.
3. Were the measurements and collection/analysis of data done in blind manner?

**Author Answer:** As this is a cross-sectional study, held at a specialized center in celiac disease treatment, the patients had the knowledge of the presence or absence of celiac disease. However, the team involved in the study was trained to perform all the steps in a blind manner. Thus, all the study was conducted in a single blind manner. We add this information in the methods section.

4. Avanutri software is validated? More details should be given and possibly references should be cited in the text.

**Author Answer:** The Avanutri Revolution® software package is in Brazilian market since 2004. This program allows the assessment of Brazilian dietary intake with effectiveness, since it contains typical food and recipes of our country. Our assessments of food intake were based on two food composition tables of our country, that is contained in the software: Brazilian Table of Food Composition - TACO and Nutritional Composition Table of Food Consumed in Brazil - IBGE. Its references were added to the text in the methodology section. Moreover, several Brazilian studies published in journals with high impact factor used this same software [9-11] and has been shown that it has all the features needed to evaluate the dietary intake of our population [12].

5. More data about pressure values, heart rate, glycaemic/lipidic profiles might be of importance regarding nutritional status of subjects; if available should be reported.

**Author Answer:** We added blood pressure (systolic and diastolic), glycaemia, total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides values in table 1.

6. Clinical history of volunteers were all negative? E.g. no thyroid pathology or autoimmune disease?
Author Answer: Yes. All studied patients, in both groups, did not present thyroid pathology, autoimmune diseases (except for celiac patients) or cardiovascular disease. In order to make it clear in the text, we added this information in the results section.

7. Some comments about higher number of females in the groups should be done, in particular with reference to a more appropriate adherence to diet or a different approach to alimentary behaviour; haemoglobin is around 13 mg/dl in both groups even if young females are the majority.

Author Answer: We thank the reviewer for the observation and as we agree with the importance of this topic, we added a paragraph about it in the discussion section.

8. I think that 6 months could be a relatively short period for a complete normalization of absorption mechanisms; authors make some comments, but a 12 months-period actually may be a better choice – some more comments are welcome.

Author Answer: We agree with this point of view and for that reason, we were very careful in designing the study, establishing that one of the inclusion criteria would be that celiac patients should be on gluten-free diet for at least 6 months, as literature shows that after this period of time there is intestinal mucosa recovery [13]. Associated with that, our results showed that celiac patients were on gluten-free diet for $1.2 \pm 0.6$ years, meaning that it is in accordance with the reviewer opinion. To let it more clearly in the text, we added a comment about it in the discussion section.