Author’s response to reviews

Title: Randomised, double-blind, placebo-controlled clinical trial of the effects of L-ornithine on stress markers and sleep quality in healthy workers

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Nutrition Journal
Dear Dr. Nehme Gabriel:

We are grateful for the opportunity to revise our paper MS: 5630901811180565 entitled “Randomised, double-blind, placebo-controlled clinical trial of the effects of L-ornithine on stress markers and sleep quality in healthy workers”, and the helpful comments of your reviewers.

We attach a version showing the tracked changes and, separately list our point-by-point responses. We feel that the comments have allowed us to improve the paper and hope you convey our gratitude to the reviewers.

Yours sincerely,

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Referee 1:

Reviewer’s report:

Major compulsory revisions

i. Method section: L-ornithine was purchased from Kyowa Hakko Bio. Does this company belong to Kirin Co Ltd? If so, this should be clarified or added as a potential conflict of interest (In previous published study using L-ornithine, the authors declared conflict of interest)

   We have included the information as follows: “In this study, we used L-ornithine monohydrochloride, a product of Kyowa Hakko Bio Company, Limited. This company is an affiliate of Kirin Company, Limited, to which the authors belong. None of the authors….”

ii. Method section, statistical analysis: why did the authors decide to include 52 patients?

   We refereed a previous study evaluating the effect of L-ornithine on physical fatigue. In the double-blind, placebo-controlled, 2-way crossover study, 17 healthy volunteers were randomized to the L-ornithine or placebo group (Nutr Res. 2008 Nov;28(11):738-43.). We decided to keep at least 23 subjects per group in this study for continuity of study and future study comparisons. We also allowed for the possibility of people dropping out, however, none did.

iii. Results section, first paragraph: even if there is no differences between two groups, the authors should add work characteristics of the study population (ie shift work…)

   Most of the study subjects were general office workers in both groups. We have included this in the Results.

iv. Results section, second paragraph: how could the authors conclude that mood state improvement was induced by L-ornithine, since they did not show any dosage? In my opinion, plasma ornithine level should be added.

   We didn’t check plasma ornithine levels in this trial. We need to confirm any correlation between plasma ornithine levels and stress markers or mood states in a future study. We have included this information in the limitations paragraph in the Discussion.
v. Discussion section: authors should be more cautious in their conclusion since they don’t have plasma ornithine level and only tendency in their statistical results. Furthermore, a limitation paragraph should be added.

   We added a paragraph explaining the limitations of this study.

Minor essential revisions
1. Abstract, background, first paragraph: suppress the two sentences about corbicula.
   We deleted the two sentences.

2. Background, fourth paragraph: reference 13 does not match (1973 is not so recent…)
   We inserted the correct reference.

3. Methods section: why did the authors decide to administrate L-ornithine for 8 weeks? Some references or an explanation should be added.
   Most previous studies have evaluated the short term effect of L-ornithine supplementation, but we were interested in the long term effects. After consideration of the seasonal effect and the volunteer’s burden, we decided on an 8 weeks study period. This has been added to the Method (“Study design”) section.

4. Discussion section: In my opinion, limits of the three tests assessing fatigue and mood states should be discussed.
   As mentioned, we have added a paragraph explaining the limitations of this study in the Discussion.

Discretionary revisions
1. Background, first paragraph: the first reference does not seem appropriate; authors should find a reference which supports the idea (ie not a review).
   We have inserted another reference.

2. Background, second paragraph: the authors should add some references about fatigue and fatigue related stress.
   We have inserted extra references
3. Methods, Study population first paragraph: physically demanding work should be defined
   
   We changed the expression to “physical work, such as carpenter or delivery person,”.
Referee 2:

Reviewer’s report:

• Major Compulsory Revisions

1 In Fig. 1 – why concentrations/ratio at zero time are zeros? What are p values on the graph? If they refer to the final point (8 weeks) as indicated, then why the asterisk is lacking in C?

   We show the means of the change from 0 weeks of each stress marker level in Fig. 1. We have included this extra information in the figure legend.

   The p values on the graph showed the results of the two-way analysis of variance, which was the first step of the statistical analysis: we have deleted these

2 Tables 2 and 4 would be better to substitute for figure panels, similar to fig. 1. Important is to start with the non-zero difference in the parameters at zero time. If I understood correct, those are given in Table 1.

   Tables 2, 3, and 4 have been changed to figure panels, similar to Fig.1.

3 Please, pay attention to the biphasic curves in Fig. 1 upon prolonged ornithine supplementation. That is, Fig 1 shows a trend of increasing the cortisol levels and ratios after initial decreases. Why there is a trend of increasing DHEA-S and initial drop of cortisol in the control group? Graphical presentation of the results of the Tables shall enable to better correlate these trends.

   The initial decreasing tendency of cortisol and DHEA-S, as objective stress markers, correlates with sleep and stress and subjective feelings of sleep and stress in the placebo group. According to your suggestion, it was easy to confirm this trend by graphical presentation.

   However, it may be that what happens just after formal ornithine and placebo intake mirrors a non-specific natural change, rather than being a unique or form of change. It is widely accepted that, on average, brief initial interventions yield outcomes similar to those with prolonged treatments, suggesting that changes could be triggered after relatively little treatment (Miller & Rollnick, 2002; Prochaska & Norcross, 2007). We have added this to the Discussion section.

• Minor Essential Revisions
1. Please, discuss the introduced dose with those which may be present in the ornithine-rich food

   We have added further information on ornithine dose from foods in the background section.

2. Lines 112-113 - Check mg of ornithine - it is different from that in line 166 – and the added mg of cellulose. Is it necessary to repeat the information in lines 112-113 and 166-167?

   We changed the expression to “Test Substance” and altered the mg of ornithine so they are the same. We moved the Methods section around: a re-named subsection, called “Test Substance” was introduced before “Study Design” and repeating information was deleted.

3. Line 153. Do you mean “... full time jobs which did not involve shift work, physically”? Correct

   We changed the expression to “excluding those who engaged in shift work or physical work...”

4 Please decipher all abbreviations, including HPA, CHR (line 227) etc

   We have added the list of abbreviations.

5 Line 267-268 – reformulate to clarify

   I have reformulated this part.

The author can be trusted to make these. For example, missing labels on figures, the wrong use of a term, spelling mistakes.

• Discretionary Revisions

1. What is the metabolic connection between GABA and ornithine?

   We have added information on the metabolic connection in the Discussion section, as below:

   “L-ornithine levels in the brain increased after oral administration of L-ornithine in mice (Kurata, 2012). Moreover, it was shown that i.c.v. injected L-arginine, the precursor of L-ornithine, increased both L-arginine and L-ornithine concentrations in the telencephalon and
diencephalon in chicks 10 min post-injection (Suenaga et al., 2008), however, the GABA content was not changed. This suggests that the sedative and hypnotic effects of L-ornithine were not due to changes in GABA synthesis (Kurata, 2011).”

2. You may consider doing functional approximations of the time-dependences (e.g., in Fig.1, or of the time dependence presented in Table 2). The difference in the approximation function/parameters strengthens significance of the differences, even if separate points do not give statistically significant results.

   Thank you for your suggestion. We analyzed the functional approximations of the time-dependences, according to your advice. However, the results were almost the same as the results shown in Fig.1 and Fig. 2. So, we considered that it might be better not to include the additional analysis in the existing figures. We deeply appreciate your suggestion though.

3 You may consider to reduce the title. The running title without technical details sounds more effective.

   We reduced the title and running title, as below:

   **Title**
   Randomised controlled trial of the effects of L-ornithine on stress markers and sleep quality in healthy workers

   **Running tile**
   L-ornithine improves stress and sleep