Author's response to reviews

Title: L-carnitine ameliorated fasting-induced fatigue, hunger, and metabolic abnormalities in patients with metabolic syndrome: a randomized controlled study

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Author's response to reviews: see over
Dear Mr. Charle Bryan S. Acuña

On behalf of my co-authors, we thank you very much for giving us an opportunity to revise our manuscript, we appreciate editor and reviewers very much for their positive and constructive comments and suggestions on our manuscript entitled “L-carnitine ameliorated fasting-induced fatigue, hunger, and metabolic turbulence in patients with metabolic syndrome: a randomized controlled study”. (ID: MS 1297770459131917)

We have studied reviewer's comments carefully and have made revision which marked in red in the paper. We have tried our best to revise our manuscript according to the comments. Please find the revised version in attached files, which we would like to submit for your kind consideration.

We would like to express our great appreciation to you and reviewers for comments on our paper. Looking forward to hearing from you.

Thank you and best regards.

Yours sincerely,

Qin Jian

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Dear Editors and Reviewers:

Thank you for your letter and for the reviewers’ comments concerning our manuscript entitled “L-carnitine ameliorated fasting-induced fatigue, hunger, and metabolic abnormalities in patients with metabolic syndrome: a randomized controlled study”. (ID: MS 1297770459131917) Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have studied comments carefully and have made correction which we hope meet with approval. The main corrections in the paper and the responds to the reviewer’s comments are as following:

Responds to the review’s comments:

Review Judit Bene:

1. Response to comment 1: The structure of the abstract does not comply with the journal’s instructions for authors.
   
   Response: Thank you for your advice, we have re-written this part according to journal’s instructions for authors.

2. Response to comment 2: The authors mentioned in the Abstract that “aspartate aminotransferase (AST) did not change significantly in the LC group after prolonged fasting” but data in Table 3 showed that this is true for ALT.
   
   Response: We are very sorry for our careless. According to your advice, we have made correction carefully. In contrast to CT group, ALT did not change significantly during fasting period in LC group.

3. Response to comment 3: Reference 11 and 12 are not relevant in the
introduction section.

Response: After careful consideration, we delete the related sentence according to your suggestion.

4. Response to comment 4: There are several references doubled in the reference list (e.g. 12,18; 21,22; 30,31; 14,31)

Response: All the repeated references had been checked and rewritten.

5. Response to comment 5: To mention the calculation formula of BMI in the “Clinical Evaluation” section is not necessary.

Response: Calculation formula of BMI in the clinical evaluation section had been deleted.

6. Response to comment 6: In Table 2 “minus” signal is missing from WC changes data. The results of lipid parameters are in Table 4, but it is mentioned in the “Change in lipid profiles” part in Table 3. The authors mentioned in the “Results” part that #-GGT concentration decreased significantly in both group, but the decrease in the CT group does not reach the significant level.

Response: We are sorry for our negligence. All mistakes mentioned above had been carefully corrected.

Special thanks to you for your good comments.

Review Gustav Dobos:

1. Response to comment 1: You proposed that “In cases where severe adverse events occurred …., the participant had to stop the treatment and his data remained in the analysis.”

Response: In fact, we viewed the side effects of L-carnitine Injection in dispensatory in detail before the study began. As far as we known, belonging to nutrition supplementation L-carnitine injection could cause two main side effects including severe gastrointestinal upset and seizure.

In the adverse reaction section, we reported that two women complained of transient nausea during the study period which was considered to be side effect of L-carnitine. All the participants accomplished study without severe
adverse reaction, so there was no drop out during the period. In addition, we meant in the article that once severe side effects occurred, the participants and data would be excluded from the study together.

2. Response to comment 2: where did they stay during fasting: in the hospital or at home?
Response: In the study, all participants stay in hospital during pre-fasting and fasting period.

3. Response to comment 3: Neither in the Trial Registry nor in the study report you defined primary and secondary outcome parameters. We conclude that your study is a preliminary pilot study.
Response: We are very sorry for our negligence of defined primary and secondary endpoint in the Trial Registry. When we design our study, body mass was design as primary outcome parameters. When we register randomised control trial, we failed to define the primary outcome and secondary outcome in the Trial Registry. However, defined outcome was noted in the research protocol which has been committed to submission in the Trial Registry.

4. Response to comment 4: how did you determine the sample size?
Response: In the previous study, it has been found that average weight loss was 0.5kg per day during the first fasting week. Thus we estimated that mean weight loss in MetS patients during fasting period was about \(-3.5 \pm 1.0\) kg, and we presumed that L-carnitine could promoted weight loss about \(-1.0\)kg during fasting period according to the previous . Pre-setting parameters followed as: \(\alpha=0.05\), power=0.1, \(\delta=1.0\)kg, \(S=0.75\), Distribution ratio= 1:1, using the Power and Sample Size Calculation. After conversion, 13 subjects were needed in each group to detect significant difference. In the event of about 15% drop-out, sample size was design about 30. (Power and Sample Size Calculation version 3.1.2, 2014 by William D. Dupont and Walton D. Plummer, Jr.)

5. Response to comment 5: Randomization: please note what software you used. Allocation details are insufficient: please add ratio and concealment. Who
generated and enrolled participants, who assigned to interventions?
Response: During the randomization, all participants were stratified into two groups by gender. Random number table was generated by SPSS 13.0 software. Randomization ratio was 1:1. During the study, Shi LY generated and preserve the randomize number in the sealed envelope. When the patients agree to participant into the trial, WU Zhi-bing was assigned to open the envelope and arrange the treatment and was responsible for intravenous admixture distribution. CAI You-jin was assigned to intravenous injection and general nursing care. Furthermore, ZHANG Jun-jie and QIU Chao-ping was allocated to clinical observation and data collection. The trial was conducted from October to December in 2012.

6. Response to comment 6: Blinding: Who was blinded and how.
Response: This was randomized, single-blinded, placebo-control clinical trial. CAI You-jin was responsible for intravenous injection and nursing care. She did not know the treatment of different patients. Wu Zhi-bing was allocated to intravenous admixture distribution. Patients were blinded from the study as result of injection with the same volume of transparent solution in two groups. Medical doctors including Zhang and Qiu who performed baseline and outcome assessments were blinded from the randomization status.

7. Response to comment 7: Results: Please note for each group the number of participants who were analyzed. As far as we can see, there were no drop outs.
Response: As we design the study, we set up in the article, we defined the period of trial was about 7-days of treatment from the beginning of per-fasting day to the last day of fasting. We defined the period of trial in the Intervention of Method section according to your suggestion.

8. Response to comment 8: Discussion: One emphasis lies on the perception of hunger and fatigue. You did not register the two items. Please explain
Response: In fact, as we design the trial, perception of hunger and fatigue were designed as indication for side effects. All the patients participating in
fasting therapy in hospital were asked to fill the fasting diary which including perception of hunger and fatigue index. As we register the trial for the first time, cardiovascular disease risk factors and hepatic enzyme were defined as the outcomes without side effects in the Trial Registry. However, as we mentioned in the introduction, perception of hunger and fatigue were included as outcomes in the study. So we are very sorry for negligence of main defect for our register.

Thank you for your good suggestion.

Review Andreas Michalsen:

1. Response to comment 1: As it stands this is more an explorative pilot study. There is no sample size calculation and no predefined primary endpoint.
   Response: When we design our study, body mass was design as primary outcome parameters. When we register randomized control trial, we failed to define the primary outcome and secondary outcome in the Trial Registry. However, defined outcome was noted in the research protocol which has been committed to submission in the Trial Registry. In the previous study, it has been found that average weight loss was 0.5kg per day during the first fasting week. Thus we estimated that mean weight loss in MetS patients during fasting period was about $-3.5 \pm 1.0$ kg, and we presumed that L-carnitine could promoted weight loss about $-1.0$kg during fasting period according to the previous. Pre-setting parameters followed as: $\alpha=0.05$, power=0.1, $\delta=1.0$kg, $S=0.75$, Distribution ratio= 1:1, using the Power and Sample Size Calculation. After conversion, 13 subjects were needed in each group to detect significant difference. In the event of about 15% drop-out, sample size was design about 30. (Power and Sample Size Calculation version 3.1.2, 2014 by William D. Dupont and Walton D. Plummer, Jr.)

2. Response to comment 2: There should be a CONSORT diagram showing the Patient flow.
   Response: In our short-term randomization controlled trial, we fail to draw a
CONSORT diagram describing patients flow. In the revised version, we add the CONSORT diagram as Figure 1.

3. Response to comment 3: How were missing data handled? Please clarify?
   Response: In our 7-day study, missing data were some body mass and scores of perception of fatigue for each day. The missing data was replaced by median of nearly points.

4. Response to comment 4: A major limitation of this study is that only one group received injections, which have a known profound unspecific or placebo-like effect. Thus, it is not surprising that all psychological outcomes are better in this group.
   Response: For the first, we stress in intervention section that participants in control group were injected with saline to eliminate bias during 7-day period. For the second, I would like to emphasize that injection may play negative effect on mood as invasive intervention. So I do not agree that injection twice a day could improve psychological outcomes. In the end, we fail to find the previous clinical report about the effect of L-carnitine on appetite. However, the benefit of L-carnitine on fatigue was validated in previous clinical studies. So we concluded from above that L-carnitine was effective on perception of hunger and fatigue.

5. Response to comment 5: It seems that despite randomization the two groups were quite different regarding blood pressure. Even though the control group was leaner they had higher blood pressure. Please clarify if there was any medication interacting.
   Response: There were 4 MetS patients in CT group and 5 in LC group under anti-hypertensive medication. During the study, we find blood pressure in 5 patients including 3 patients with hypertension increased in LC group versus one without hypertension in CT group (data not shown). As shown in table 2, changes in blood pressure between groups showed no significant different because baseline values play vital part. In the control group, all participants showed decreasing trend in blood pressure during fasting period. Hypertensive
patients had poor blood pressure control in CT group as compared with that in LC group. As we analyze in details, it has been found that blood pressure of hypertensive patients increasing during period had better blood pressure control in LC group than that in CT group before study. All hypertensive patients were asked to take anti-hypertensive medicine as before. So we suggested that L-carnitine may be responsible for blood pressure stabilization during fasting period. We have supplemented the detail of patients taking anti-hypertension medication.

6. Response to comment 5: The discussion regarding the role of carnitine in fat burning and weight loss should be more detailed and more critical.
Response: According to your suggestion, we added another two related experimental research articles and one clinical study report about the effect of L-carnitine on weight loss. In fact, there was no clinical study validated the effective of carnitine on weight loss whether during calorie restriction or during aerobic training till now.

7. Response to comment 6: Also the authors should discuss more in detail their findings regarding mood and psychological well-being during fasting in relation to the existing abundant literature.
Response: Before our study, we had reviewed all related article about fasting therapy. Modified fasting therapy which could applied to treat chronic pain syndrome and arthritis was able to achieve mood enhancement. However, the influence of L-carnitine on MetS patients during modified fasting therapy was the main part of our article. And the mood and psychological well-being were not the primary outcomes as we defined during the study. So we did not think it was appropriate to stress the positive effect of modified fasting therapy on mood.

8. Response to comment 7: Finally, the literature should be better checked and re-evaluated. For example Ref 26 seems to be not appropriate.
Response: We are very sorry for our negligence of checking reference. According to your suggestion, we carefully re-evaluated the article and made
correction.

Special thank to you for your good comments.

Minor changes:
The title of author unit of Wu Zhi-bing was finally confirmed, which was minor modified for different translation.
The telephone numbers of correspondence author has been changed.
The authors’ contribution also has been supplemented.