Author's response to reviews

Title: Effects of L-carnitine supplementation on oxidative stress and antioxidant enzymes activities in patients with coronary artery disease: a randomized, placebo-controlled trial

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Author's response to reviews: see over
Dear Dr. Nagaraj Nagathihalli,

Thank you very much for your review of our manuscript entitled “Effects of L-carnitine supplementation on oxidative stress and antioxidant enzymes activities in patients with coronary artery disease: a randomized, placebo-controlled trial”. We have carefully addressed the comments of the reviewers (please see the following pages) and we have incorporated their suggestions in the revised manuscript. Please review the revised version of our manuscript. If you have any questions, please contact me. Thank you very much for considering our revised manuscript for publication in your journal.

Sincerely,

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Manuscript (ID: 1462232731301742) “Effects of L-carnitine supplementation on oxidative stress and antioxidant enzymes activities in patients with coronary artery disease: a randomized, placebo-controlled trial”. The answers of reviewers’ comments follow:

Reviewer #1:
Q1. Your study is presented in a straightforward, understandable style; the English is good. I can find nothing wrong with your procedures or conclusions. In your abstract, you should note that plasma levels of LC and MDA, and erythrocyte antioxidant activities have been measured.
A1. Thank you for the reviewer’s comments. We have addressed the descriptions in the Abstract section; please see page 2, lines 21-24.

Q2. With respect to your discussion, I am not so sure that L-carnitine's direct oxidant scavenging activity is of much significance. You might revise this to note that carnitine, by acting as a buffer for excessive acetyl groups in mitochondria, may be able to decrease mitochondrial superoxide production during hypoxia or substrate excess. The antioxidant effect you have detected may be of greater importance in ischemic tissues.
A2. We agree with the reviewer’s comments and have added the note that “LC could be acting as a buffer for excessive acetyl groups in mitochondria, decreasing mitochondrial superoxide production during hypoxia or substrate excess, especially in the ischemic tissues“ in the Discussion section; please see page 9, lines 170-173.

Reviewer #2:
Q1. If there were any cardiovascular events or deaths please report
A1. There was no cardiovascular event or death report during and the end of the study. Thank you for the reviewer’s comments. This is an important point with regard to the safety of LC supplement, we have added this point in the Discussion section; please see pages 9-10, lines 192-193.
Reviewer #3:

Q1. Hypothesis and novelty: The question of antioxidant capacity in CAD is novel and the manuscript is relatively concise and clear. However the aim and hypothesis are not sufficiently sharp. What is the reason for testing 12 weeks LC supplementation in patients with CAD? It seems that changes in antioxidant activity would apply in healthy subjects as well and perhaps with a more favorable signal to noise ratio? If tested in CAD patients would it then not be appropriate to continue LC supplementation for a longer period of time and evaluate clinically relevant parameters?

A1. Thank you for the reviewer’s comments.

(1) We agree with the reviewer’s concern, it should be test for a longer period time to evaluate the beneficial effects of LC in CAD patients. This short-term intervention is one of the limitations of the study; please see the Discussion section (page 10, lines 203-205). However, after 12 weeks of LC supplementation, CAD subjects had significantly increased their antioxidative capacity. Further study is needed to follow up the levels of antioxidative capacity for a longer period of time after supplementation.

(2) A recent study was conducted by Cao et al. (2011), they administrated liquid LC (2000 mg) to the healthy subjects (n = 12). The results showed that healthy subjects had significantly increased their antioxidant capacity after supplementation. As a result, Cao et al. suggested that LC may be useful as a dietary supplement for chronic illnesses involving excessive oxidative stress, such as CAD. However, little information has been published about the effect of LC on antioxidant status in CAD patients. To our knowledge, this is the first study to investigate the antioxidative effect of LC supplementation in patients with CAD. Thus, it is worth knowing whether LC could be a dietary supplement for CAD patients. Please see Background section (page 3, lines 42-45).

Q2. Results are really only table 2 and even so the authors have chosen not to depict pre and post values? Data should also be graphically presented with clear representation of pre and post values.

A2. Thank you for the reviewer’s comments. Figure 2 presented the pre and post values of LC, oxidative stress, and antioxidant enzymes activities. Table 2 presented the delta values (week 12-0) of LC, oxidative stress, and antioxidant enzymes activities. We have described in detail the values of these parameters in the Results section. Please see page 7, lines 124-139.
Q3. The study would benefit greatly from investigation of more mechanistic parameters.
A2. Thank you for the reviewer’s comments. This clinical study was focused on the antioxidant effect of LC supplements in CAD patients. Therefore, we measured an oxidative stress maker (MDA) and antioxidant enzymes activities (CAT, SOD, and GPx) to assess the antioxidative capacity after LC supplementation. However, we agree with the reviewer’s comment, further study is needed to measure more mechanistic parameters to understand the antioxidative mechanism of LC in CAD patients. We have added this point in the Discussion section. Please see page 10, lines 206-208.

Q4. The safety profile of LC supplementation is not thoroughly addressed.
A4. Thank you for the reviewer’s comments. We also measured the values of hematological entities [including blood urea nitrogen (BUN), creatinine, glutamic oxaloacetic transaminase (GOT), and glutamic pyruvate transaminase (GPT)], but data were not presented in the study. The data are shown below (Table 1). The values of BUN, creatinine, GOT, and GPT were not significantly different after supplementation or between the two groups. In addition, there was no cardiovascular event or death report during or at the end of the study. We have added these points in the Discussion section; please see pages 9-10, lines 189-193.

Table 1 Values of hematology after supplementation

<table>
<thead>
<tr>
<th></th>
<th>Placebo(n = 19)</th>
<th>LC(n = 20)</th>
<th>P values $^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Week 0</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BUN (mM)</td>
<td>13.4 ± 7.8</td>
<td>14.2 ± 5.4</td>
<td>0.25</td>
</tr>
<tr>
<td>creatinine (µM)</td>
<td>106.1 ± 44.2</td>
<td>114.9 ± 26.5</td>
<td>0.33</td>
</tr>
<tr>
<td>GOT (IU/L)</td>
<td>23.7 ± 7.9</td>
<td>21.6 ± 7.3</td>
<td>0.30</td>
</tr>
<tr>
<td>GPT (IU/L)</td>
<td>21.3 ± 14.8</td>
<td>17.0 ± 8.9</td>
<td>0.24</td>
</tr>
<tr>
<td><strong>Week 12</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BUN (mM)</td>
<td>13.0 ± 6.4</td>
<td>13.7 ± 4.1</td>
<td>0.43</td>
</tr>
<tr>
<td>creatinine (µM)</td>
<td>106.1 ± 35.4</td>
<td>114.9 ± 35.4</td>
<td>0.13</td>
</tr>
<tr>
<td>GOT (IU/L)</td>
<td>25.2 ± 12.9</td>
<td>22.5 ± 7.2</td>
<td>0.73</td>
</tr>
<tr>
<td>GPT (IU/L)</td>
<td>20.1 ± 11.0</td>
<td>15.2 ± 6.4</td>
<td>0.13</td>
</tr>
</tbody>
</table>
Q5. The discussion section is highly speculative and the link between antioxidant capacity in RBC’s and atherosclerosis/CAD is only addressed in vague terms. A hypothetical link between RBC conditions and endothelial/myocardial conditions should be described (or investigated!) in more detail in order to justify the coupling between antioxidants, LC and CAD of this study.

A5. Thank you for the reviewer’s comments. Decreased RBCs antioxidative enzymes might accelerate the development of atherosclerosis [1]. Increased RBCs antioxidant enzymes activities can provide a protection against oxidative damage to the endothelial cells [2]. In the present study, there was a significant positive correlation between the levels of LC and antioxidant enzymes activities after supplementation. LC was found to be an effective antioxidant agent in cardiovascular disease models and prevent endothelial dysfunction through its antioxidant property [2-4]. As a result, it seems clear that LC has a protective effect against CAD, which could be ascribed to its antioxidant capacity. We have added this description in the Discussion section; please see page 8, lines 151-158.

References

Q6. The background for using the MDA assay as an indicator of oxidative damage is not touched upon. Could one assess ROS production and oxidative damage in a more direct manner?

A4. Thank you for the reviewer’s comments. In the present study, we used MDA assay as an indicator of oxidative stress marker because MDA is easily quantifiable in clinical practice and shows promise as a clinical biomarker. MDA is generated in vivo via peroxidation of polyunsaturated fatty acids. Studies showed that MDA could predict progression of CAD and carotid atherosclerosis at 3 years [1, 2]. However, MDA assay does not have a functional impact on the pathophysiology of CAD [2]. We agreed with the reviewer’s opinions, further study is needed to measure more direct indicators of oxidative damage, such as nitrotyrosine, myeloperoxidase (MPO), or oxidized low density lipoprotein (ox-LDL) levels. We have added these points in the Methods section (page 5, lines 85-88) and Discussion section (page 10, lines 206-208).

References


Q7. Are the mitochondria central in the assessment of antioxidants and oxidative damage? Mitochondria are only mentioned once throughout the entire paper.

A7. Thank you for the reviewer’s comments. LC is an endogenous substance that acts as a carrier for fatty acid across the inner mitochondrial membrane for the substance β-oxidation cycle and ATP production. The present study demonstrated that LC is a potent antioxidant in CAD patients. As a result, we suggest that LC could be acting as a buffer for excessive acetyl groups in mitochondria, decreasing mitochondrial superoxide production during hypoxia or substrate excess, especially in the ischemic tissues. We have added these points in the Discussion section (page 9, lines 170-173).

Q8. Generally the manuscript should be improved grammatically. Quality of written English: Needs some language corrections before being published.

A8. Thank you for the reviewer’s comments. The manuscript has been re-edited by American Journal Experts (AJE). The editorial certificate of AJE has shown below.
Reviewer #4:

Q1. Regarding to this, the authors should explain clearly the real contribution of the results of this investigation, since there are previous studies evaluating the effect of long term administration of LC (using the same dose or higher) on the antioxidant activities in patients under different circumstances.

A1. Thank you for the reviewer’s comments.

(1) This clinical study was focused on the antioxidant effect of LC supplements in CAD patients. However, most of the studies were examined the antioxidant properties of LC in cell and animal models. A recent human study was conducted by Cao et al. (2011), they examined the antioxidative activities after administrated liquid LC (2000 mg) to the healthy subjects (n = 12). The results were showed that healthy subjects had significantly increased their antioxidant capacity after supplementation. As a result, LC may be useful as a dietary supplement for chronic illnesses involving excessive oxidative stress, such as CAD. However, little information has been published about the effect of LC on antioxidant status in CAD patients. To our knowledge, this is the first study to investigate the antioxidative effect of LC supplementation in patients with CAD. Thus, it is worth knowing whether LC could be a dietary supplement for CAD patients. Please see the Background section (page 3, lines 42-45).

(2) With regard the dose of LC (1000 mg/d), the Ministry of Health and Welfare in Taiwan recommends a daily dietary intake of no more than 2000 mg of LC. As a result, we tested a dose of 1000 mg/d in CAD patients and expected that the dose of LC (1000 mg/d) could be a dietary supplement for daily use. Based on the results of this study, we suggest that LC might be a useful dietary supplement for CAD to protect against excessive oxidative stress. Please see the Discussion section (page 9, lines 179-183).

Q2. There are recent evidences about the negative effects of LC administration: recent studies suggest that dietary LC may accelerate atherosclerosis via gut microbiota metabolites, complicating the role of LC supplementation in health (Johri et al., Nutr Metab Cardiov Dis 2014). Did the authors consider these possible negative effects of LC chronic administration?

A2. Thank you for the reviewer’s comments. Recent work has suggested that dietary LC might accelerate atherosclerosis via gut microbiota metabolites, complicating the role of L-C supplementation in health. However, most of the studies showed benefits of LC supplementation for cardiovascular disease. We considered that the need for LC supplementation in CAD patients might be dependent on the status of LC in the body. Thus, LC is considered a "conditionally essential nutrient. Animals and human studies have shown that the content of LC was low in acute myocardial infarction and chronic heart failure [1,2]. In our opinion, it should be recommended that CAD patients with lower levels of LC and higher oxidative stress take LC supplements to increase their LC status and
anti-oxidation capacity. Our data suggest that a dose of 1000 mg/d is safe for CAD patients. However, our study was designed using daily LC supplements for 3 months only. Longer intervention studies are needed to understand and establish the beneficial effects of a high dose of LC in patients with CAD. We have added these viewpoints in the Discussion section (page 10, lines 195-201, and lines 203-205).

References


Q3. Did the authors consider any additional medication in the patients? If so, in which manner this medication could interact with LC supplementation?

A2. Thank you for the reviewer’s comments. Medications such as acenocoumarol (sintrom) [1, 2], thyroid hormone [3], and warfarin (coumadin) [4] might interact with LC. LC might increase the effectiveness of acenocoumarol and warfarin, and decrease the effectiveness of thyroid hormone. As a result, patients currently being treated with these drugs were excluded from the study. We have added this point in the Methods section; please see page 4, lines 61-62.


Q4. Page 7, lines 118-128: this paragraph is not clear. The authors explain the results at 12 weeks and at the end of treatment, but it is supposed that the duration of the treatment was 12 weeks. Please clear this statement.

A4. Thank you for the reviewer’s comments. We have described in detail of these values of the parameters in the Results section. Please see page 7, lines 124-139.