Author’s response to reviews

Title: (R)-α-Lipoic acid inhibits fructose-induced myoglobin fructation and the formation of advanced glycation end products (AGEs) in vitro

Authors:

Hardik Ghelani (h.ghelani@westernsydney.edu.au)
Valentina Razmovski-Naumovski (v.naumovski@westernsydney.edu.au)
Rajeswara Pragada (profprrau@gmail.com)
Srinivas Nammi (S.Nammi@westernsydney.edu.au)

Version: 1 Date: 18 Nov 2017

Author’s response to reviews:

18 November 2017

Dr Seung Min Oh and Dr Soledad Guyala
BMC Complementary & Alternative Medicine

Thank you very much for the evaluation of the paper (BCAM-D-17-01108):

(R)-α-Lipoic acid inhibits fructose-induced myoglobin fructation and the formation of advanced glycation end products (AGEs) in vitro

Hardik Ghelani, Valentina Razmovski-Naumovski, Rajeswara Rao Pragada, Srinivas Nammi

Enclosed please do find the revised manuscript with changes highlighted in RED font. As listed below, we have considered all the points raised by the reviewers during this revision, and thus do hope that you and the referees consider the paper now acceptable for publication in BMC Complementary & Alternative Medicine.
We thank you again in advance for your consideration.

With kind regards,

Srinivas Nammi

Reviewer 1:

Comment 1:

Although the authors have demonstrated that incubations of myoglobin with fructose (in the absence of ALA) reproduce the expected effects of fructation and their consequences (i.e., increases in the relative levels of AGE, increases in fructosamine and protein carbonyl contents, reductions in thiol groups, and increases in the release of iron from myoglobin), it cannot be affirmed that the protective effects of ALA in this in vitro system are directly related to the protection of myoglobin against the post-translational modifications caused by fructose or other intermediates generated during incubation. ALA itself may, for example, (i) absorb the fluorescence emitted by AGEs at 460 nm; (ii) absorb at the wavelengths used for measurement of protein carbonyl and free thiol groups. Therefore, some controls should be presented: (i) performing all investigations of this study with incubations of myoglobin + ALA, in the absence of glucose; (ii) absorption spectra of ALA, to investigate whether such compound does not have interferences in critical wavelengths used in assays, such as 460 nm (AGE emission), 375 and 412 nm (wavelengths used in protein carbonyls and free thiol assays, respectively).

Authors’ response:

The authors appreciate the reviewer for critical review of the manuscript advising to include “myoglobin + ALA” group that prompted the authors to include this additional control group in the ongoing studies related to this project. Although this group is not presented in this manuscript, the non-interference of ALA on absorbing fluorescence emission at 460 nm and on absorption wavelengths at 412 nm and 375 nm is could be explained and justified based on published literature. For example, in a previously reported glycation study, ALA alone did not interfere with fluorescence emission at wavelengths from 410 nm to 462 nm (Suzuki et al. Free Radic Res Commun 1992; 17: 211-217). In addition, studies on spectral analysis reported that ALA alone did not cause any absorption at wavelengths in UV-visible region from 300 nm to 475 nm (Koricanac et al. Journal of Serbian Chemical Society 2007; 72: 29-35; Godlewska et al. Journal of Analytical Methods in Chemistry Volume 2015, Article ID 535387, 7 pages)
Comment 2:

It is not correct to name "myoglobin + fructose" incubation as "disease control". In vivo, there is a complex interplay of molecular and biochemical mechanisms during the promotion of the complications associated with glycation/fructation and AGE that are not restricted to the processes observed using an in vitro approach, as the case of this study. Please use "fructation positive control" or other denomination close to an in vitro modification.

Authors’ response:

The authors agree with the comment of the reviewer. As suggested, the representative group names “normal control” and “disease control” are now changed to “non-fructated control” and “fructated control” respectively in the text and figures of the revised manuscript.

Comment 3:

Considering the in vitro approach used here, this study lacks of evidences about the ability of ALA to attenuate or prevent late glycation/fructation events. Incubations of myoglobin with GK peptide or investigation of crosslinking/protein aggregation must be performed. For reference, please see study by Hsia et al. (Journal of Functional Foods, 21: 406-417, 2016).

Authors’ response:

The authors agree with the reviewer that the study lacks further evidence about the effect of ALA on late glycation events. The present work is a preliminary study in the umbrella of a larger project that aims to investigate ALA intervention on different glycation intermediates (such as methylglyoxal and glyoxal)-mediated glycation events that lead to AGEs production and on AGEs-mediated protein cross-linking and cellular signalling pathways. These identified limitations are the topics of our current research and the results will be communicated as a separate publication in near future. Information pertaining to the limitations of this study and future work is now added in the discussion section (Page 17).

Reviewer 2:

Comments to authors:

This work is very interesting and has a big scientific potential. I found some issues which should be corrected before publication. In abstract the aim of study should be given. Introduction section is very interesting but is too long and should be shortened. Some editorial bugs, revision of text
should be performed. The carbonylation colorimetric assay is not variable. I suggest using the ELISA method according to Alamdari. Means ± SEM should be changed to means ± SD. How did Authors estimate the possibility of using one-way ANOVA? In the discussion section, should a section about ALA bioavailability be added in accordance with the concentrations used in this study.

Authors’ response:

The authors are pleased with the positive feedback on the scientific potential of the work. As suggested, the objective of the study is now added in the abstract section (Page 2). The introduction section is also shortened by moving a few sentences to the discussion section (Pages 16, 17) as advised by the reviewer. The manuscript text and the changes made are proof-read for any grammatical and typographical errors and corrected. Although the ELISA method developed by Alamdari et al. (2005) has been used to measure protein carbonyls, the colorimetric method developed by Levine et al. (1994) has also been widely used as a cost-effective method to measure detectible levels of protein carbonyls in assay systems similar to our study by various investigators (Uchida et al. PNAS 1998; 95: 4882-4887; Jariyapamornkoon et al. BMC Complement & Alternat Med 2013; 13: 171; Tupe et al. J Food Sci Technol 2015; 52: 1911-1923; Ashraf et al. PLoS One 2015; 10; e0130630; Tube et al. Pharm Biol 2016; 55: 68-75; Meeprom et al. Molecules 2013; 18: 6439-6454). As suggested, the means ± SEM is changed to means ± SD in the results section and in the figures. In the discussion section (Pages 14, 15), information on ALA bioavailability to justify the studied concentrations is now added.