Reviewer’s report

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

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Reviewer: Amanda Martins Baviera

Reviewer's report:

Ghelani and collaborators explored the anti-fructation potential of alpha-lipoic acid (ALA) using and in vitro approach, incubating myoglobin with fructose and in the absence and presence of ALA at different concentrations and during different periods.

Authors found that ALA: (i) inhibited the formation of AGE (evaluated by the fluorescence intensity of the incubation medium), (ii) reduced the fructosamine levels, (iii) reduced the protein carbonyl content and increased the thiol content in myoglobin, (iv) reduced the iron release from myoglobin. Authors concluded that ALA had anti-glycation potential and emphasized that ALA can be useful as a supplementation to prevent the diabetic complications related to glycation process and AGE toxicity.

Overall this paper was written with a sound purpose, showing very interesting findings to substantiate evidences of the beneficial effects of ALA against the post-translational modifications of proteins that can participate in the onset of diabetic complications or AGE-related disturbances. However the manuscript should be rewritten to address the following issues.

(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 can not 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).

(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.

(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).

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

No

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Yes

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