Reviewer's report

Title: L-Citrulline increases hepatic sensitivity to insulin by reducing the phosphorylation of serine 1101 in insulin receptor substrate-1

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Reviewer: Elisa Keating

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The authors aimed to study the potential of L-Citrulline in ameliorating insulin sensitivity. To do this, they studied the effect of L-Citrulline upon insulin signalling cascade in rat hepatoma cells and in the liver of a rat model of the metabolic syndrome. The hypothesis is well defined and may add knowledge to the field, however there are several concerns.

Major Compulsory Revisions

1) The methods are not sufficiently detailed. Cell culture methods must rigorously indicate the concentrations of the test compounds used (e.g., 100 uM Citrulline was also used and it is omitted). It must be described in detail in which experiments the cells were treated with Dex/cAMP. It is not clear whether in this protocol the cells were exposed during 6 h or during 1 h to L-Cit.

2) The biological soundness of the work may be compromised by the extremely high amount of L-Citrulline used both in cell culture and in animal experiments. Can the authors explain the choice of the concentrations in each set of experiments? Is there any correspondence of these doses to the physiological or even to a pharmacological situation in the human?

3) In animal assays there is no indication about the mode of L-Cit administration. Was it in the drinking water, was it a special L-Cit supplemented chow? Did the animals have free access to water and food? Why did the authors choose an 8-week treatment?

4) Did the authors measure blood arginine as a biomarker of increased citrulline status?

5) The description of statistical analyses is quite insufficient. The authors should describe in detail in which sets of data they used t-test (eventually for comparison between 2 groups) or analyses of variance with post-hoc tests such as Tukey’s.

6) There is no reference to the sample size (n) concerning cell culture data. How many replicates were performed in each experiment and how many experiments? This should also be added to the table and to the figure legends. Still regarding the sample size, please explain why the animal groups contained 7 animals each, but western blot analysis only refer to n=5.

7) The models used should be clearly described (the description of H4IIE cells – tissue of origin, etc). Additionally, the choice of the models (cells and animals) used should be clearly presented and justified. Given that the main objective was
to study the effect of L-Cit in reducing insulin resistance (or ameliorating insulin sensitivity), the exposure of H4IE cells, which respond normally to insulin, to L-Cit does not seem to inform much about the capacity of L-Cit to revert insulin resistance. Can the authors discuss this issue?

8) In figure 1B, data concerning the effect of L-Cit alone on PEPCK mRNA levels is missing.

9) There is no information on the concentration of L-Cit used in Figure 3. In Figure 3C insulin alone does not seem to increase p-IRS ser1101, as it is later stated in the discussion (line 283, page 13). Please correct this contradiction. How do the authors justify this absence of effect upon the phosphorylation of IRS on ser1101?

10) In page 11, lines 233 and 234, the authors state that there are no differences in the phosphorylation of IRS-1 Ser 307 and 612 between treatment groups, however quantitative analysis for these western blottings is not presented in figure 4. Please add the relevant graphs to figure 4.

Minor Essential Revisions

1) Dose of L-Cit in rat chow is presented in the abstract as 2g/Kg/day and in the methods section it appears as a percentage. Please correct and explain the conversion between them.

2) Line 83, page 5: replace the phrase: “improving insulin resistance” with “reducing insulin resistance” or “improving insulin sensitivity”

3) Line 202, page 10: replace “gluconeogenetic” with “gluconeogenic”

4) Line 204, page 10: replace the word insulin is misspelled

5) Animal results are presented in figure 4, however they are always remitted to figure 3. Please correct.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests.