ANSWERING REVIEWERS

Santiago, June 45, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 10838-revised.doc).

Title: T_3-induced liver AMPK signaling: redox dependency and upregulation of downstream targets

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The manuscript has been improved according to the suggestions of reviewers:

Reviewer- 1:

This is a part of the consecutive work by the authors. Their thesis and presented data has been approved by their own numerous publications. I appreciate the achievement of the research group. Readers who know their work want probably to get something new in this paper. Hence, it would be better that new findings are more emphasized. I suggest the authors consider this point. Another point that I concern is in their basic concept. It can be interpreted that endogenous oxidative stress is necessary to induce antioxidation mechanisms. To my knowledge, there are far more oxidation factors than antioxidants in the usual aerobic condition. Therefore, I think that antioxidant factors including AMPK are already induced sufficiently without T3-mediated preconditioning. I'd like to get a hint to solve this question in the present paper.

Answer to comment-1: In order to emphasize the new findings reported, the following two paragraphs were inserted in the revised version of the work:

in page 12-lines 13-16:

"Data presented indicate that T_3-induced ROS production, revealed by higher 8-isoprostane levels in liver and plasma, have a causal role in upregulating rat liver AMPK signaling, resulting in FAO enhancement to support energy-demanding processes such as T_3-PC or tissue repair[5-7] (Fig. 4).",

and in page 13-lines 2-5:

"In agreement with this contention, T_3 was recently shown to stimulate hepatic FAO coupled with the induction of autophagy, a stress-related process degrading cellular components to produce FAs to generate ATP or amino acids to synthesize proteins for cell survival[39].",

and new reference [39] was added to the reference list, and the rest were renumbered.

Answer to comment-2: The statement that "there are far more oxidation factors than antioxidation mechanisms in the usual aerobic condition" is correct. However, AMPK is not considered a direct antioxidant factor, but rather an adaptive mechanism to favor energy production under stress conditions. AMPK-induced ATP availability by T_3 is necessary to support preconditioning mechanisms, which include the synthesis of antioxidant proteins, enzymes, and reduced glutathione.

Reviewer-2:

This is a detailed report on the mechanisms of T3 in mediating AMPK signalling. The authors covered a range of early and late kinases in detail and showed that T3 mediate AMPK signalling in a redox dependent manner. I appreciate the effort and the authors put into their work and the idea of using T3 for preconditioning. I would like the authors to address these points before publishing, 1) In fig 1B, NAC alone seems to increase AMPK mRNA level by 10 times compared to controls, it would be good to at least discuss this or perform a western to show p-AMPK level in the NAC group compared to the controls. 2) Authors should show some functional changes (eg. autophagy ability, LC3-II) of T3, vs control, NAC, T3 + NAC.

Answer to comment-1: In fact, NAC alone was able to increase AMPK mRNA levels by 6.6-fold over control values. However, according to the statistical analysis performed using ONE-way ANOVA and the Newman-Keuls, test, the difference between these two groups is not significantly different, as stated in Figure 1B.

Answer to comment-2: According to the aims of the work, functional changes regarding T_3 versus controls, NAC, and NAC + T_3 are presented in terms of the oxidative stress status attained in the different groups studied and that establish the causal role of ROS on liver AMPK activation. In addition, as indicated for reviewer-1 in the second part of the answer to comment-1, recent work by Sinha et al. (J Clin Invest 2012; 122: 2428-2438) reported that thyroid hormone stimulates hepatic FA oxidation via activation of autophagy, which is in agreement with our proposal of FA oxidation enhancement through activation of AMPK signaling, as an alternate mechanism. Thus the following paragraph was introduced in page 13-lines 2-5:

"In agreement with this contention, T_3 was recently shown to stimulate hepatic FAO coupled with the induction of autophagy, a stress-related process degrading cellular components to produce FAs to generate ATP or amino acids to synthesize proteins for cell survival[39].",

And reference by Sinha et al. was added as number 39 to the reference list, and the rest were renumbered.

Additional changes (shown in yellow in the revised version)**:**

a) Page 11-line 22: in this line, "PGC-1α" was shown by mistake in the original version of the
" in the revised version.
ect abbreviation of the journal in the revised version.

ilable".

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