**Title: Protective role of adiponectin in a rat model of intestinal ischemia reperfusion injury**

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**Dear Editors and Reviewers,**

Thank you for your letter and for the reviewers' comments concerning our manuscript entitled "The protective role of adiponectin in a rat model of intestinal ischemia reperfusion injury" (Manuscript #17366). Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have studied comments carefully and have made correction which we hope meet with approval. Revised portion are marked in the paper. Below are our responses (in blue) to the reviewers' specific comments (in black).

**Reviewed by 03259111**

The title is short, conclusive reflecting the content of the study. The abstract is clear with obvious delineation between the various parts. Materials, Methods and statistics: the count of the animals is not included, with defective comment on the the sample size and the statistical data. the references are not up to date

Thank you for your valuable suggestions. There are 5 rats in each groups and weight of them are 180~230 g. (page 6, line 157).And we have updated some references.(page 16, line 446-582)

**Reviewed by 03069152**

As mentioned in the manuscript that adiponectin receptor 1 and receptor 2 have no changes in the protein level, the author should discuss how the recombinant adiponectin reacted with the cell membrane to further activated the AMPK/HO-1 pathway.

Thank you for your suggestions. Adipo R (AdipoReceptor) is involved in various biological

effects of adiponectin, and is closely related to obesity, diabetes, and cardiovascular disease[1]. In different tissues, the expression levels and the regulating factors of Adipo R are different. Adiponectin receptor expression is elevated in colorectal carcinoma, but not in gastrointestinal stromal tumors[2]. The expression of placental adiponectin is increased during pregnancy in the rat, while Adipo R2 shows the opposite pattern. In this study, protein levels of Adipo R1 and Adipo R2 in the I/R and I/R + adiponectin groups did not change significantly, while p-AMPK and HO-1 increased in the I/R + adiponectin group compared to the I/R group which are consistent with the results of Chen[3] and Cheng et al[4]. However, it is unclear whether it is Adipo R1, Adipo R 2, or their combination which have an effect on the protein level of p-AMPK and HO-1. Perhaps the function of Adipo R is not dependent on the protein level in intestinal I/R injury. We intend to conduct experiments on this mechanism in our next study.(page 14,ling381-397)

Abstract: result, "Adiponectin was down-regulated in the intestinal tissues", tissues or serum? And "adipoectin" should be change into "adiponectin" through the manuscript. "attenuated the protective effects of adiponectin on intestinal I/R injury" on should be change to "against".

Thank you for pointing out these issues. We have corrected them throughout the manuscript.

Materials and Method: "Establishment of the rat model of intestinal I/R injurya" is it correct? "approximate 10 cm segment of the small intestine was harvested for follow-up experiments", the position of small intestine should be mentioned. "intraperitoneal injection of recombinant adiponectin of different concentration (0.25, 0.5, 1mg/kg)" while in the result section "which were pre-treated with adiponectin of different concentrations (0.25μM, 0.5μM, and 1μM)" the dose of adiponectin should be corrected. "To explore the pathway which may involve in the protection effects of adiponectin, the IR+adiponectin (1mg/kg) received Compond C or Snpp injection" the origin, dose and route of administration of Compond C and Snpp should be mentioned. The antibodies message of adiponectin receptor 1 and receptor 2 should be added in "western blot".

Thank you for point out these issues, and the corrections are as below:

(1)"Establishment of the rat model of intestinal I/R injurya" should be "Establishment of the rat model of intestinal I/R injury"(page 6, line 152)

(2) position of small intestine harvested： approximate 10 cm segment of the small intestine 10cm from the appendix was harvested for follow-up experiments(page 7, line 169)

(3) different concentrations of recombinant adiponectin should be 0.25, 0.5, 1mg/kg(page 7, line 177; page 10, line 261)

(4) the information of Compound C and Snpp are as follows(page 7,181-183):

| Name | Origin | Route of Administration | Dose |
|---|---|---|---|
| Compound C | Millipore(171260) | intraperitoneal injection | 20mg/kg |
| SnPP | Sigma (P8293) | intraperitoneal injection | 50 micro mol/kg |

(5) Antibodies of adiponectin receptor 1 and receptor 2 were from abcam and catalogue numbers are ab70362 and ab77612, respectively.(page 8,line 217-218)

All the corrections have been made accordingly in the manuscript.

Discussion: “Increased levels of ischemia markers such as MDA”, MDA is not a ischemia marker, just a oxidative stress marker.

Thank you for your valuable advice. We have corrected this sentence like this: “Increased levels of oxidative stress marker such as MDA”(page 12,line 338)

A injury score should be raised to the Fig.1C.

The evaluation of intestinal mucosal lesions showed significantly increased villous injury scores in rats with I/R injury (1, 2, 3, 6, and 12 h after reperfusion) compared with that in the sham group (Figure 1C and D).(page10,line 252-255)

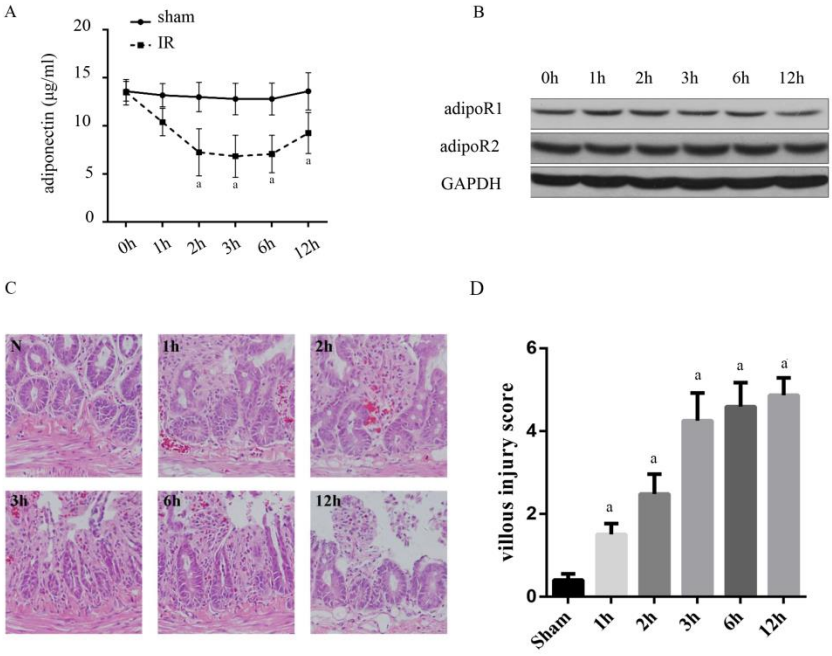


**Figure 1 Adiponectin levels in rats in the sham and I/R injury groups**. (A) ELISA was used to determine the adiponectin serum levels in the sham and I/R injury rats. Data are presented as mean ± SD obtained from 5 independent experiments. (B) Western blot analysis of the expression of adiponectin receptor 1 and adiponectin receptor 2 in the small intestine of rats with I/R injury. (C) HE staining of the small intestine at different time points after I/R injury. Necrosis was the major damage after intestinal I/R. N: colon tissue in normal control rat. [a] $P<0.05$ *vs* sham.

Fig. 3A the p-AMPK western band should be corrected as the "Compound C" seems no effect on inhibition of p-AMPK compared with IR group.

Thank you for your valuable suggestion. In fact, IR + Compound C group in our manuscript should be IR + adiponectin + Compound C group which is clearly stated in the Materials and Methods section. Through the comparison with IR + adiponectin group we can draw a conclusion that Compound C inhibited the phosphorylation of AMPK. We are so sorry about the confusion caused by us and all the mistakes about this issue have been corrected accordingly both in the text and in the figures.

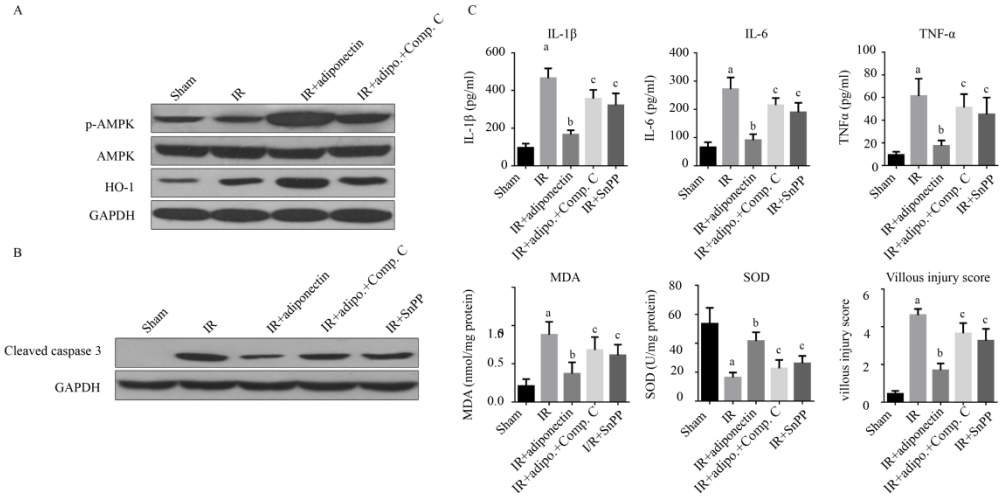


**Figure 3 AMPK inhibition attenuated I/R injury**. (A) Western blot analysis of the expression of p-AMPK and HO-1. (B) Western blot analysis of the expression of cleaved caspase 3. (C) The serum levels of IL-1β, IL-6, TNFα, MDA, and SOD and the villus damage score. Adipo: adiponectin; Compo. C: Compound C; Compound C: AMPK signaling pathway inhibitor; Snpp, HO-1 inhibitor. The data are expressed as mean ± SD obtained from 5 independent experiments. $^{a}P<0.05$ *vs* sham, $^{b}P<0.05$ *vs* IR, $^{c}P<0.05$ *vs* IR + adiponectin.

We appreciate the time and effort of the reviewers and the editorial staff and thank you for the opportunity to resubmit this manuscript for publication.

Best regards!

Yours sincerely,

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**References**

1 Sowers JR. Endocrine functions of adipose tissue: focus on adiponectin. *Clin Cornerstone* 2008; **9**(1): 32-38, 39-40[PMID:19046738

2 Williams CJ, Mitsiades N, Sozopoulos E, Hsi A, Wolk A, Nifli AP, Tseleni-Balafouta S, Mantzoros CS. Adiponectin receptor expression is elevated in colorectal carcinomas but not in gastrointestinal stromal tumors. *Endocr Relat Cancer* 2008; **15**(1): 289-299[PMID:18310295DOI: 10.1677/ERC-07-0197].

3 Hui C. Relevant mechanism of adiponectin activating the AMPK signaling pathway to regulate lipid metabolism in bovine hepatocytes, 2013.

4 Cheng CF, Lian WS, Chen SH, Lai PF, Li HF, Lan YF, Cheng WT, Lin H. Protective effects of adiponectin against renal ischemia-reperfusion injury via prostacyclin-PPARalpha-heme oxygenase-1 signaling pathway. *J Cell Physiol* 2012; **227**(1): 239-249[PMID:21412771DOI: 10.1002/jcp.22726].