Dear editors and reviewers,

Thank you for giving us the opportunity to submit a revised draft of the manuscript "Antagonizing adipose tissue-derived exosome miR-103-hepatocyte PTEN pathway alleviates autophagy in non-alcoholic steatohepatitis (NASH): a trans-cellular crosstalk" for the consideration of publication in the World Journal of Gastroenterology. We appreciate the time and effort that you and the reviewers dedicated to provide feedback on our manuscript and are grateful for the insightful comments on our paper. We have revised our article according to the comments of the reviewers.

Reviewer 1: Authors used an animal model to generate the NASH using a high fat diet, but authors do not show any data validating this model: in other words, NASH analysis must be shown in the animals submitted to the model diet. The design of the animal model is unclear: When miR_NC and mir-103-ANTA were injected (13 weeks according to the methods section), were the animals still on diet? The experimental groups are not clearly defined: control and model, and then these splitted in 2 groups?: Control diet + miR_NC; Control diet+miR103 ANTA; model diet + miR-NC and model diet + mir103ANTA, is that correct? If so, graphs should be labeled correctly to allow a proper reading of the manuscript. Otherwise, authors should carefully explain the experimental groups. I am looking forward to the explanations that will allow me to continue the peer review process.

A: Thank you for your suggestions. In fact, we validated the NASH mice model by serum ALT, AST, triglyceride, cholesterol and other serological indicators, as well as liver lipid deposition, inflammation degree and other pathological manifestations by H-E staining. I am sorry that we did not state this clearly in the results section in the previous version. Thanks to the reviewer's comments, we have supplemented this part in the results section ("First, we successfully constructed animal models of NASH. Compared with the control, ALT, AST, TG, and CHOL were increased in NASH mice (Figure 1A). Hepatocyte ballooning, inflammatory cell infiltration, and hepatic lipid accumulation were observed in the livers of NASH mice (Figure 1B, 1C)").

Besides, in the previous version we did not explain the grouping process clearly in the method section, the reviewers were confused. In the revised version, we have rewritten the method section for grouping ("C57BL/6 mice were routinely fed a high-fat diet for 12 weeks to establish the NASH animal model. According to different treatments, they were initially divided into the control group (12% kcal fat, 66% kcal carbohydrate, 22% kcal protein) and the model group (60% kcal fat, 20% kcal carbohydrate, 60% kcal protein, 20% kcal protein). Starting from the 13th week, 40 mg/kg miR-NC-anta and miR-103-anta were injected into the mice from the model group (dissolved in 0.2 mL normal saline) through the tail vein every 2 days thrice to construct miR-NC-anta model group and miR-103-anta model group. The control and model groups were injected with blank normal saline thrice (n=10 in each group)"). When miR_NC and mir-103-anta were injected, the animals were still on NAFLD diet. Our group is not "the Control diet + miR-NC, Control diet + miR-103-ANTA, model diet + miR-NC and model diet + miR103-ANTA" the four groups. Actually, miR-NC and miR-103 anta were only used to treat NASH mice to explore the therapeutic effect of miR103 inhibition on NASH. Please see the detailed description in the method section ("C57BL/6 mice were routinely fed a high-fat diet for 12 weeks to establish the NASH animal model. According to different treatments, they were initially divided into the control group (12% kcal fat, 66% kcal carbohydrate, 22% kcal protein) and the model group (60% kcal fat, 20% kcal carbohydrate, 60% kcal protein, 20% kcal protein). Starting from the 13th week, 40 mg/kg miR-NC-anta and miR-103-anta were injected into the mice from the model group (dissolved in 0.2 mL normal saline) through the tail vein every 2 days thrice to construct miR-NC-anta model group and miR-103-anta model group. The control and model groups were injected with blank normal saline thrice (n=10 in each group)").

Reviewer 2: The study demonstrated that miR-103 expression was increased in NASH mice. Moreover, inhibition of miR-103 alleviated NASH via inhibition of autophagy/PTEN pathway. miR-103 is an adipose-tissue derived exosomal miR. Authors concluded that miR-103 can inhibit autophagy in hepatocytes and thus, regulates the development of NASH (at least in mouse model in vivo). The study is

interesting and addressed an important pathology. However, there are several issues which require amendments. 1. Abstract does not reflect the findings properly. Results section of the Abstract does not report which main indicators of autophagy were assessed. How did authors confirm the role of autophagy? Which markers were used? I suggest re-writing the Abstract/Results section to reflect clearly how authors confirmed the role of miR-103 in autophagy. For instance, Western blotting results with LC3 may be mentioned. 2. Abstract: the last sentence is confusing ("More importantly, the elevation of miR-103 in the liver of NASH mice is partly due to adipose tissue exosome secretion and integration, which also partially explains the mechanism of obesity leading to NAFLD"). This conclusion is confusing. I do not think that it is possible to claim this link. This should be re-phrased and/or more experimental data is required to support this statement. 3. miR-103 was found linked to G protein-coupled estrogen receptor 1 (GPER1) (see this paper Fang T, Li J, Wu X. Shenmai injection improves the postoperative immune function of papillary thyroid carcinoma patients by inhibiting differentiation into Treg cells via miR-103/GPER1 axis. Drug Dev Res. 2018 Nov;79(7):324-331. doi: 10.1002/ddr.21459.). This is a very promising and interesting findings which can be mentioned next to the problem of insulin sensitivity demonstrated in NAFLD patients. Fatty lever problem was recently linked to menopause (see this paper DiStefano JK. NAFLD and NASH in Postmenopausal Women: Implications for Diagnosis and Treatment. Endocrinology. 2020 Oct 1;161(10):bqaa134. doi: 10.1210/endocr/bqaa134.), and thus, potentially, with estrogen signaling pathway. Authors may need to mention this in the Introduction and Discussion section. 4. All abbreviations should be deciphered. 5. Methods: very few citations in this section. Please cite more relevant papers to direct readers towards the published papers which used similar techniques and described them properly (full description). 6. Figure 1: IHC images should be enlarged. It is hard to see the cellular composition with this magnification. 7. Fig.2D, 3A, 5c,d, 6c– should be enlarged; it is hard to see the cells and what is going on there. Good job with fig.4 – very clear presentation of data. Authors should do the same with Figure 1,2 , 3 , 5 and 6 IHC images. 8. Figure 7 should be improved. The conclusion is not shown. Inhibition of autophagy

by miR-103 somehow influences the autophagosome formation (inhibits or stimulates? It is not shown on your diagram). Suggestively, the number of autophagosomes should be decreased – however, it is not clearly shown – there is a green arrow – what does it mean? Clarify. 9. Conclusions should be linked to the NAFLD and insulin resistance more clearly.

1. We have revised the results section of the abstract based on the reviewer's comments ("The expression of miR-103 was increased in NASH mice, compared to the control, and inhibition of miR-103 could alleviate NASH. The results of the dual-luciferase reporter assay showed miR-103 could interact with PTEN. MiR-103-anta decreased p-AMPKa, p-mTOR, and p62 but increased the protein levels of PTEN and LC3-II/I and the number of autophagosomes in NASH mice. Similar results were also observed in NASH-like cells, and further experiments showed PTEN silencing inhibited the effect of miR-103-anta. AT derived-exosome miR-103 aggravated NASH and increased the expressions of p-AMPKa, p-mTOR, and p62 but decreased the protein levels of PTEN and LC3-II/I and the number of autophagosomes in mice").

2. We have revised the conclusion of the abstract according to the reviewer's comments to make our conclusions more rigorous ("AT derived-exosome increased the levels of miR-103 in the liver, and miR-103 aggravated NASH. Mechanically, miR-103 could interact with PTEN and inhibit autophagy").

3. Thank you very much for the reviewer's suggestions on our discussion section. We have enriched the content of the discussion section according to the reviewer's opinions ("NASH was linked to menopause, and miR-103 was found to be linked to G protein-coupled estrogen receptor 1 (GPER1). Therefore, the estrogen signaling pathway is the potential mechanism where miR-103 promotes NASH").

4. All abbreviations have been explained in the revised version.

5. In the Methods section, we add references to support some important methodological methods (Reference 13, 14, 15).

6. We have further adjusted our pictures and increased the resolution of the pictures according to the comments of the reviewers (Figure 1).

7. We have further adjusted our pictures and increased the resolution of the pictures according to the comments of the reviewers (Figure 2, 3, 5, 6).

8. We have revised Figure 7 according to the comments of the reviewers. We hope that the modified picture 7 can show our results more clearly.

9. Since our paper did not further verify the effect of miR-103 on insulin resistance, we did not add or subtract the content related to insulin resistance in the results any more ("To sum up, our study confirms the important role of miR-103-PTEN - autophagy axis in NASH, and the elevation of miR-103 in the liver of the NASH model is partly due to hepatocyte absorption of AT derived-exosomes, which also partially explains the underlining mechanism of obesity leading to NASH").