

Dear Dr. Ma:

Thank you very much for your decision letter and advice on our manuscript (Manuscript 44583), entitled “Ursodeoxycholic acid ameliorates hepatic lipid metabolism in LO2 cells by regulating the AKT/mTOR/SREBP-1 signaling pathway”. We also thank the reviewers for their constructive and positive comments and suggestions.

Accordingly, we have revised the manuscript in accordance with the reviewers' comments, and carefully proofread the manuscript to minimize typographical, grammatical, and bibliographical errors. All amendments have highlighted in yellow color in the revised manuscript. In addition, the point-by-point responses to the comments are listed below this letter.

This manuscript has been edited and proofread by American Journal Experts. I hope that the revision is acceptable for publication in your journal, and I look forward to hearing from you soon.

Yours sincerely,

Lei Ye

Response to Reviewer 1:

Major comments:

1. Title: 'lipid metabolism' should be changed to reflect the main result of the study.

Response: Thanks for raising this critical issue. We have changed 'lipid metabolism' in the title to 'hepatic lipid metabolism in LO2 cells', which can appropriately reflect the main result of the study. We have made the changes in the revised manuscript accordingly (Page 1, Lines 7).

2. Figure 2: There is no significant change between OA group and UDCA group. Thus UDCA did not reduced triglyceride contents. How do you can explain the phenotype? Author should logically revise the discussion.

Response: Thank you for the insightful comment. As indicated in Figure 2 , Triglyceride contents is no significant change between OA group and UDCA group. UDCA-treatment can not be a significant reduction of Triglyceride level in OA-induced cells. We have revised discussion .The Necessary changes have been made in the revised manuscript (Page 12, Lines28-30; Page 13, Lines1-4).

3. Please check statistics and present in figures. They are very confusing and difficult interpretation.

Response: Thank you for your kind comment. I have checked statistics in figures. We have made the changes in the revised figures accordingly (Page 21-24, Figure1-4).

4. UDCA directly regulate cholesterol metabolism. Did you determine SERBP-2?

Response: Thank you very much for your attention and consideration. Before the experiment, we studied a lot of relevant literatures. We know that CRT2 modulates triglyceride synthesis via regulation of SREBP1 maturation. By contrast, CRT2 did not modulate the activity of SREBP2, which is consistent with the lack of cholesterol accumulation. Therefore, we have not detected SERBP-2, only studied research on the SREBP-1.

5. Rewrite clearly abstract to summarize and reflect the results.

Response: Thank you for your thoughtful suggestion. The necessary changes have been made in the manuscript accordingly (Page 2, Lines 2-12; Page 4, Lines1-7).

Minor comments :

1. Figure 2-D y axis: insert unit.

This correction has been made in the revised manuscript. (Figure 2-D)

2. Figure 3 & 4: What is mean $hp < 0.01$?

This correction has been made in the revised manuscript (Figure3, Figure4). $^b p < 0.01$, vs ctrl group; $^c p < 0.01$ means vs model group.

3. Unify unit in entire manuscript. (ex. mL vs. ml)

This correction has been made in the revised manuscript. (Page 7, Lines 26; Page 8, Lines15; Page 8, Lines18; Page9, Lines18; Page9, Lines24).

4. Correct typing errors (ex, CO₂, oil red O....) and leaving space.

This correction has been made in the revised manuscript. (Page 7, Lines 25; Page 8, Lines7; Page 8, Lines16; Page9, Lines19). Leaving space has been modified in the revised manuscript.

Response to Reviewer 2:

1. Overall the manuscript is well written and the authors have well organized and excellently collected, provided and discussed their findings, however there are some typing and writing errors in the manuscript. Authors should correct them. In the literature, the effects of UDCA on NAFLD in the clinic, and especially on liver histology is limited. The authors should discuss this issue in the discussion.

Response: Thank you for your good evaluation and kind suggestion. We have made corrections according to your comments. We have revised and added this issue in the discussion. The necessary changes have been made in the manuscript accordingly (Page 14, Lines 1–7).

Response to Reviewer 3:

1. The manuscript is well-written but somewhat disappointing by the lack of ambition. This is surprising because their findings are limited to a simple cell model. Considering the conclusions, a more precise and extensive approach to the AMPK/mTOR driven pathways should be considered.

Response: Thanks for your thoughtful suggestion. Our findings expected to provide evidence that UDCA have the effect of ameliorating lipid metabolism. However , our research has some limitations and further studies

should be performed using animals and further vitro research with agonists and inhibitors of mTOR as tools. We have found my shortcomings in our research. We will follow your advice to improve my scientific research level and make more achievements in our future work.

Point-by-Point Responses

1. Please provide language certificate letter by professional English language editing companies (Classification of manuscript language quality evaluation is B).For manuscripts submitted by non-native speakers of English, please provided language certificate by professional English language editing companies mentioned in 'The Revision Policies of BPG for Article'.

Response: We have provided language certificate letter by professional English language editing companies.

2. Audio coretip: In order to attract readers to read your full-text article, we request that the author make an audio file describing your final coretip, it is necessary for final acceptance. Please refer to Instruction to authors on our website or attached Format for detailed information. The accepted formats are mp3 or wma.

Response: We have provided audio file describing my final coretip.

3. Please provide the decomposable figure of figures, whose parts are all movable and editable, organize them into a PowerPoint file, and submit as "Manuscript No. - image files.ppt" on the system.

Response: We have organized figures into a PowerPoint file, and submitted as "Manuscript No. 45583 image files.ppt" on the system.

4. Your manuscript should be prepared with Word-processing Software, using 12 pt Book Antiqua font and 1.5 linespacing with ample margins.

Response: We have used 12 pt Book Antiqua font and 1.5 lines pacing with ample margins in the revised manuscript.

5. Please revise and perfect your manuscript according to peer-reviewers' comments. Please upload the required files on the system.

Response: We have revised and perfected my manuscript according to peer-reviewers' comments.

6. Title

Response: We Accepted the Suggestions of Reviewer 1, and modified the title accordingly in the revised manuscript. (Page 1, Lines 6-7).

7. Authors' names:

This correction has been made in the revised manuscript (Page 1, Lines 9-10).

8. Author contributions:

It has been added in the revised manuscript. (Page 1, Lines 22-24).

9. Corresponding author:

This has been revised in the revised manuscript. (Page 2, Line 13).

10. Abstract Background :

This has been added in the revised manuscript. (Page 3, Lines 3-12).

11. Abstract Results:

The necessary changes have been made in the manuscript accordingly (Page 3 , Lines 1-7).

12. Key words:

These corrections have been made in the revised manuscript (Page 4 , Line 13).

13. Core tip:

The necessary changes have been made in the manuscript accordingly (Page 4 , Lines15-22; Page4 , Lines25-26).

14. Discussion:

Response: We have referred to the suggestion of Reviewers. Accordingly, discussion has been added in the revised manuscript. The necessary changes have been made in the manuscript accordingly. (Page 11, Lines 20-26; Page12, Lines 23-29)

15. Article Highlights

Response: These have been added in the revised manuscript. (Page 13, Lines 1- 30 ; Page 14, Lines 1-29).

16. References:

We have checked and revised the repeated references. We have made the changes in the revised manuscript accordingly. (Page14 - Page19). Reference NO.14 changes to NO. 13(Page 6, Line 4); Reference NO.15 changes to NO. 14(Page 6, Line 4); reference NO .16 changes to NO. 15(Page 6, Line 7); reference NO.17changes to NO .16(Page 6, Line 9); reference NO .18 changes to NO .17(Page 6, Line 11); reference NO. 19 changes to NO .18(Page 6, Line13); reference NO.20 changes to NO. 19(Page 9, Line 25); reference NO.21 changes to NO .20(Page 10, Line12); reference NO. 22 changes to NO .21(Page 10, Lines 12; Page12, Line12); reference NO.23 changes to NO .22(Page 10, Line26); reference NO. 24 changes to NO .23(Page 10, Line26); reference NO .25 changes to NO .24(Page 10, Line29); reference NO.26 changes to NO. 25(Page 11, Line9); reference NO .26 changes to NO .25(Page 11, Line9); reference NO. 27 changes to NO .26(Page 11, Line9); reference NO .28 changes to NO. 27(Page 11, Line11); reference NO .29 changes to NO .28(Page 11, Line11); reference NO. 30 changes to NO .29(Page 11, Line15); reference NO. 31changes to NO .30(Page 11, Line17); reference NO .32 changes to NO. 31(Page 11, Line29); reference NO. 33 changes to NO.32(Page 11, Line29); reference NO .34 changes to NO. 33(Page 11, Line30); reference NO .35 changes to NO .34(Page 12, Line4); reference NO .36 changes to NO. 35(Page 12, Line4); reference NO. 37changes to NO. 36(Page 12, Line6); reference NO. 38 changes to

NO .37(Page 12, Line8); reference NO. 39 changes to NO. 38(Page 12, Line8); reference NO .40 changes to NO. 39(Page 12, Line10).

17.Figure:Please don't include abbreviations in the title of the figure/table.Please explain all the abbreviations in the figure/table legends: full name (abbreviation).Please explain all the abbreviations of each figure/table under each piece of figure/table legends.Please don't include any *, #, ...in your manuscript; Please use superscript numbers for illustration; and for statistical significance, please use superscript letters.

These corrections have been made in the revised manuscript. (Figure 1-4) (Page21 – Page23).

18. “lipid metabolism” should be replaced with “ hepatic lipid metabolism”. This occurs several places in the manuscript.

These corrections have been made in the revised manuscript (Page1, Line6; Page 4, Lines 13, 23, 29; Page 5, Lines 9, 11, 29; Page 6, Line 17; Page 9, Lines 5, 17, 20; Page 10, Lines 25, 29; Page 11, Line19).

19. “OA-exposed” should be replaced with “OA-induced”. This occurs several places in the manuscript.

These corrections have been made in the revised manuscript .(Page8, Line11; Page9, Line 9; Page 10, Line 19).

20. “ml” should be replaced with “mL”. This occurs several places in the manuscript.

Response: These corrections have been made in the revised manuscript .(Page6, Line27; Page7, Lines 14,17; Page 8, Line 21).

21. “5%CO₂” should be replaced with “50mLcells/mL”. This occurs several places in the manuscript.

Response: These corrections have been made in the revised manuscript (Page6, Line 26; Page7, Lines 14, 15).

22. “signalling” should be replaced with “signaling”. This occurs several places in the manuscript.

Response: These corrections have been made in the revised manuscript (Page1, Line7; Page10, Lines 6, 14, 23; Page12, Lines17, 19).

23. “oil-red” should be replaced with “oil red”. This occurs several places in the manuscript.

Response: These corrections have been made in the revised manuscript (Page7, Lines 4, 7).

24. “in LO2 cells” should be added in the manuscript. This occurs several places in the manuscript.

These corrections have been made in the revised manuscript(Page1, Line 6 Page4, Line 29)

25. Statistical significance is expressed as $aP < 0.05$, $bP < 0.01$ ($P > 0.05$ usually does not need to be denoted). If there are other series of P values, $cP < 0.05$ and $dP < 0.01$ are used, and a third series of P values is expressed as $eP < 0.05$ and $fP < 0.01$.

These corrections have been made in the revised manuscript. (Page8, Lines 24, 29; Page9, Lines 12, 29; Page10, Lines 3, 4,19)