To,
Dr. Fang-Fang Ji,
Science Editor, Editorial Office,
World Journal of Diabetes

Subject: Submission of the revised manuscript No. 39507

Dear Sir,
Thank you very much for accepting our manuscript for review in your journal, and directing the revision. Attached please find the revised manuscript for your kind consideration. We have replied to the comments of the Hon. Reviewers and Editor, which are appended to this letter. We hope that you will find the revised manuscript suitable for publication in your esteemed journal.

As per the reviewer's direction, the title of the article is changed to "Coagonist of GLP-1 and glucagon receptors ameliorates kidney injury in murine models of obesity and diabetes mellitus".

As per our initial communication, this paper is submitted for WJD Best Paper Award in response to the invitation from your office, with Dr. Vishal Patel as the contesting author.

Regards,

Amit Joharapurkar
Ahmedabad, India

10-05-2018

SPECIFIC COMMENTS TO AUTHORS

This is an excellent manuscript describing the results of an experimental research in mice with regards to the treatment with a coagonist of GLP-1R. The treatment of diabetes mellitus with GLP-1R agonists has been introduced only some years ago, with excellent results.

MAJOR CONCERN: In the abstract and in Material & Methods the authors note that the doses of the coagonist used were 150 ugr/kbw, but in the table 2 the doses are 300 ug/kbw. Please can you clarify this important methodological issue ?.

Reply: The dose of coagoinst is corrected in table 2.

MINOR CONCERNS: - The terms GLP-1 and GCGR mat be completely described the first time, before the introduction of the initials.

Reply: The full form of GLP-1 was elaborated when it appears first time.

-INTRODUCTION: 3rd file, please eliminate "the":...were closely associated and together ... - RESULTS: after the text of figure 1: ... "Feeding" high fat diet (not "feeing" fat diet).

Reply: The typographical error in result section was corrected.

- I personally find very well selected the molecules described to study the oxidative stress and the inflammatory status. In the same way, the techniques used to describe the glomerular and tubulo-interstitial lessions are very well selected and the lessions very well described.

DISCUSSION: But due to the relevant findings of this research you may add some comments on the limitations of the study, - i.e. 10 individuals in each group of research animals-.

Reply: Limitations of the study is added in the discussion sections as per review's suggestion.

On the other hand, and taking into account the applicability of this research to the clinics, you should be able to add some comments on the possible translation of these findings to the daily practice, because these experimental results are in agreement with some recent studies, such as LEADER, SUSTAIN-6 or AWARD-7, among others.

Reply: Translations value of the current study is added in the discussion section.

PEER-REVIEW REPORT

SPECIFIC COMMENTS TO AUTHORS

I have carefully read this new review manuscript. This is a preclinical study using 3 separate murine models. My major questions are summarized below:

1) Abstract does not summarize the 3 murine models.

Reply: Details of all three murine models has been added in the abstract.

2) Introduction should propose a hypothesis and then list specific aims to study the hypothesis.

Reply: Proposal of the hypothesis to study coagonist in renal dysfunction was added in introduction.

3) First sentence in Materials and Methods: the authors either need to provide either a reference for or validation studies for their contention that they are studying a coagonist.

Reply: The reference for validation of coagoinst for target engagement was inserted in the Materials and Methods section.

4) The table in Materials and Methods provides "Primer sequences" but does not presently aid in summarizing why the authors chose specific products for RT-PCR. In describing the RT-PCR methods, please consider using either "Semi-quantitation of the mRNAs" or "Normalization of the mRNA levels was performed".

Reply: The purpose of selected genes was added in Materials and Methods section along with normalization method.

5) In the Discussion, please consider adding a Cartoon that summarizes the proposed pathway.

Reply: The cartoon is added to the manuscript as Figure 5.

6) In the Discussion, there is no description of limitations of these studies; for example, protein malnutrition can alter blood creatinine levels and body weight; RT-PCR gene expression studies may reflect only "accidental transcription" since protein expression has not been studied.

Reply: The limitation of the study has been included in the discussion section.

Minor Issues: 1) The title is not fully accurate; please consider elimination of "diabetes-induced" and please consider after "kidney injury" adding "in murine models of obesity and diabetes mellitus".

Reply: As per reviewer's suggestion, the Title has been changed to "Coagonist of GLP-1 and glucagon receptors ameliorates kidney injury in murine models of obesity and diabetes mellitus"

2) Abstract, Results: consider in sentence starting with "Lipogenic" adding "expression" after each of the two "gene".

Reply: The sentence is corrected.

3) Abstract, Conclusion: Coagonist did not function by “restoring” obesity.

Reply: The statement is corrected.

4) In Core tip, the second sentence is difficult to understand. Please consider after “alleviates” “biochemical and histopathological findings of nephropathy in HFSTZ and db/db mice”.

Reply: The sentence is modified as per review’s suggestion.

5) Core tip last sentence: do the authors mean “coagonist is effective”. The authors need to proof read their manuscript. There are other sentences missing verbs. The first sentence of the Introduction should probably read “Diabetes mellitus is often associated with”.

Reply: The sentences were modified and the entire manuscript was checked and modified for these mistakes.

6) Last sentence of Introduction: do the authors mean “function in models of obesity and diabetes-induced”.

Reply: The last sentence in introduction is modified to “In the current study, we investigated the effect of coagonist of GLP-1R and GCGR in on renal dysfunction in a model of obesity and diabetes-induced renal dysfunction”

7) Materials and Methods: there is an incomplete address for Qiagen. In Materials and Methods consider specific labels for: Model 1. Renal dysfunction; Model 2. Streptozotocin-high-fat; Model 3. Genetic model of diabetes. Please provide a reference for the streptozotocin method. Then, “based of body weight”: which ranged from what to what?

Reply: The details are updated in Qiagen. The model number is included in the Material and Method sections. The reference for STZ-induced diabetes was added in

the manuscript. The randomization details of body weight and glucose is included in the manuscript.

8) Obese mice were of what age?

Reply: Age of obese mice is added in Table 2 legends.

9) Tissue sections were of what thickness?

Reply: Thickness of section (5 μM) is added in text.

10) Table in Results: Kidney weight is not in grams. Do the authors have any prior validation studies to demonstrate absence of edematous changes such as dry weight determination or result per mg protein?

Reply: The unit of kidney weight is corrected to mg in Table 2. Since the kidneys were processed immediately for histological analysis and gene expression studies, we did not process them for protein or dry weight determination. We do understand that this would have added important information, and accept as a limitation of our study.

11) Figure 1 in Results: this Figure needs to be able to stand alone and so the authors must be certain that all abbreviations are described in the Figure; p values should be shown in this Figure.

Reply: Abbreviations is included in the all the figures.

12) Table 3 in Results: this Table needs to be able to stand alone and so the authors must be certain that all abbreviations are described in the Table.

Reply: Missing abbreviations are added to Table 3.

13) Figure 3 in Results needs to be able to stand alone and so it should be clear that mRNA levels have been normalized by secondary analysis of beta-actin expression.

Reply: Figure 3 is changed as per reviewer's direction.

14) Figures 4 in Results should be able to stand alone and so the authors must be certain that all abbreviations are described in the Figure.

Reply: Missing abbreviation is added to Figure 4.

15) Discussion paragraph 1: do the authors mean “in expression of lipogenic genes”.

Reply: Yes, and word “expression” is included.

16) Discussion paragraph 2, line 1: do the authors mean “Most obese individual never”.

Reply: Yes, we have added reference that supports this statement.

17) In Discussion, the authors should consider noting that this preclinical study is an important initiation for translational research in this field of study.

Reply: Importance of preclinical research and its translational value is included in the last para of discussion.

Editors Comments

1. Institutional review board statement

Please offer signed pdf format.

Reply: Attached.

2. ***Biostatistics***

Please offer signed pdf format.

Reply: Attached and also mentioned in material and methods section.

3. ***Conflict-of-interest statement- upload for all authors.***

Reply: Attached.

4. ***Institutional animal care and use committee statement.***

Please offer signed pdf format.

Reply: Attached.

5. ***Animal care and use statement.***

Reply: Attached.

6. ***Audio Core Tip***

Please offer the audio core tip, the requriment are as follows:

Reply: Attached.

7. ***ARTICLE HIGHLIGHTS***

The guidelines for writing and formatting Article Highlights are as follows:

1 Research background

The background, present status, and significance of the study should be described in detail.

2 Research motivation

The main topics, the key problems to be solved, and the significance of solving these problems for future research in this field should be described in detail.

3 Research objectives

The main objectives, the objectives that were realized, and the significance of realizing these objectives for future research in this field should be described in detail.

4 Research methods

The research methods (*e.g.*, experiments, data analysis, surveys, and clinical trials) that were adopted to realize the objectives, as well as the characteristics and novelty of these research methods, should be described in detail.

5 Research results

The research findings, their contributions to the research in this field, and the problems that remain to be solved should be described in detail.

6 Research conclusions

The following questions should be briefly answered:

What are the new findings of this study?

What are the new theories that this study proposes?

What are the appropriate summarizations of the current knowledge that this study provided?

What are the original insights into the current knowledge that this study offered?

What are the new hypotheses that this study proposed?

What are the new methods that this study proposed?

What are the new phenomena that were found through experiments in this study?

What are the hypotheses that were confirmed through experiments in this study?

What are the implications of this study for clinical practice in the future?

7 Research perspectives

What experiences and lessons can be learnt from this study?

What is the direction of the future research?

What is/are the best method/s for the future research?

Reply: Added to the manuscript.