RESPONSES TO REVIEWERS' CONCERNS

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Alterations in gut microbiota during remission and recurrence of diabetes after duodenal-jejunal bypass in rats

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Dear editor,

Thank you very much for your letter and for the reviewers' comments concerning our manuscript entitled "Alterations in gut microbiota during remission and recurrence of diabetes after duodenal-jejunal bypass in rats". Those comments are very valuable and helpful for revising and improving our paper. Based on the comments and suggestions, careful modifications have been made to the original manuscript. A revised manuscript with the correction sections marked in highlight was uploaded for easy checking/editing purpose.

We hope the following point-by-point responses and the new revision of the manuscript will meet the editor's and reviewers' requirements for considering this manuscript for publication in *World Journal of Gastroenterology*.

Reviewer (1):

Dear Reviewer,

We sincerely thank the reviewer for your suggestions on our study. We believe that it could make the manuscript more precise. The manuscript has been modified according your suggestions:

1. The high-fat diet and low-dose streptozotocin-treated rat or mice was still widely used as a diabetes model, and relevant research was published on *Nature Medicine* in 2015 [1]. To avoid of misleading readers, the low-dose streptozotocin-treatment has been mentioned in *ABSTRACT, CORE TIP* and *INTRODUCTION.* The specific procedure of inducing diabetes in rats is described in *MATERIALS* and *METHODS.* The discussion on the inference that recurrence of diabetes after DJB is due to insulin resistance is insufficient. This inference is established based on the comparison between DJB-RC and DJB-RM groups. T2DM is a metabolic disorder characterized by insulin resistance and dysfunction of pancreatic beta-cells [2]. Compared with DJB-RM rats, DJB-RC rats exhibited comparable secretion of insulin after gavage while re-impaired insulin sensitivity as represented by a higher values of HOMA-IR. Therefore, we concluded that the recurrence of diabetes after initial remission in DJB rats was due to the re-deterioration of improved insulin sensitivity. The particular description can be found in *DISCUSSION.*
2. Our research group has been committed to uncover the mechanisms of diabetes remission after metabolic surgeries over the past decade. In our previous studies, the diabetic rats exhibited different outcomes after metabolic surgeries, for example, some rats achieved completely remission after surgeries, while some rats achieved initial remission while underwent subsequent recurrence. These differences have also been reported in some clinical studies. It makes us and other surgeons confused why it happened. This study is just designed to explore the intrinsic mechanisms of these different outcomes after metabolic surgeries. The particular description has been expounded in the third section of *DISCUSSION.*

3. Fifteen days after induction of diabetes, a low-residue diet was administered to the rats in sham group and DJB group from 48 h preoperatively to 72 h postoperatively. DJB or sham surgery was performed under anesthesia with 10% chloral hydrate (3 mL/kg). All of the surgeries were completed within 3 days. The above description has been added to the *Surgical techniques* section.
4. We are so sorry that we have not described statistical analyses clearly in this manuscript. Body weight, energy intake, AUC_{OGTT}, HOMA-IR, TBAs and LPS were evaluated using one-way analysis of variance (ANOVA) followed by ***Bonferroni post hoc comparison***. Insulin and total GLP-1 concentrations after glucose gavage between the groups were analyzed by mixed model ANOVA followed by ***Bonferroni post hoc comparison***s. Statistical differences between two groups were evaluated based on ANOVA followed by ***Bonferroni post hoc comparison*** rather than simple ANOVA.
5. The description of the microbiome analyses was modified in the section of *16S rDNA-based study of gut microbiota* as follows: Because host metabolism can be influenced by the biological activity of microbiota in the colon, we focused our study on the colonic microbiota. At 12 weeks after surgery, all rats were narcotized with 10% chloral hydrate (3 mL/kg). A colonic segment of three- centimeter was ligatured and removed from enterocoelia. The colon was incised longitudinally, then we collected the colonic faeces and stored them at −80°C. All operations were performed under aseptic conditions. Genomic DNA of colonic microbiota was isolated based on the protocol of an E.Z.N.A. Soil DNA kit (Omega, Norcross, GA, USA). The method to amplify the V4 region of microbial 16S rDNA g by PCR has been reported previously. We use Illumina MiSeq platform (BGI Technology, China) to sequence the amplified V4 region. The raw data were filtered to eliminate the adapter pollution and low quality and to obtain clean reads. Then paired-end reads with overlap were merged to

tags, which were clustered, at 97% sequence similarity, to operational taxonomic unit (OTU). By using Ribosomal Database Project (RDP) Na, e Bayesian Classifier v.2.2, we assigned taxonomic ranks to OTU representative sequence. At last, the different species screening tests were analyzed based on OTU and taxonomic ranks.

The 16S rDNA-sequencing focuses on the prokaryote, and the 18S DNA-sequencing focuses on the eucaryon. In this study, we employed the 16S rDNA-sequencing, so eukaryotic tissues were not analyzed.

6. In this study, we only observed a similar trend of LPS to the trend of the relative abundance of Escherichia_coli. The alterations in LPS may be the result of combined effects of gut microbiota, gut barrier function and host immunity. We could not demonstrate the exact causal relationship between LPS and Escherichia_coli, which should be verified in further study. The statement has been issued in the fifth section of *DISCUSSION*.
7. It is defective that we did not explain the meaning of "Energy intake" clearly. We apologize to reviewers for our cursoriness. "Energy intake" represented calorie content in the food intake in this study. We have replace "Energy intake" by "calorie content in the food intake (calorie intake)". No statistic difference in calorie intake was observed among the sham, DJB-RC and DJB-RM groups in this study. However, it is a pity that the calorie content in faeces was not measured. So, the calorie absorbed from food intake could not be calculated. To avoid misunderstanding, we have added that statement as one of limitations in this study to the eighth section of *DISCUSSION*.

Thanks for your considerations again, and we are pleased to explain any question about the manuscript further.

REFERENCES

1 **Duca FA,** Côté CD, Rasmussen BA, Zadeh-Tahmasebi M, Rutter GA,

Filippi BM, Lam TK. Metformin activates a duodenal Ampk-dependent pathway to lower hepatic glucose production in rats. *Nat Med* 2015; **21:** 506-511 [PMID: 25849133 DOI: 10.1038/nm.3787]

2 **Gerich JE.** The genetic basis of type 2 diabetes mellitus: impaired insulin secretion versus impaired insulin sensitivity. *Endocr Rev* 1998; **19:** 491-503 [PMID: 9715377]

Reviewer (2):

Dear Reviewer,

Thanks for your positive comments on the manuscript. We have summarized all abbreviations appeared in the manuscript in *Material and Methods.* The principal of method of 16s rDNA-based study of gut microbiota has been described with detail in *Material and Methods.*

We believe that your suggestions will help the readers understanding the manuscript well.

Reviewer (3):

Dear Reviewer,

We sincerely thanks for your kind words for the manuscript. The manuscript has been modified according to the comments from all reviewers and reviewed by the language editor again. We will explain any question about the manuscript.

Finally, we appreciate for editors' and reviewers' work, and hope that the revision will meet with your requirements.

Sincerely,

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