

## Round 1

Dear editor,

We sincerely thank the reviewers for thoroughly examining our manuscript entitled “Depletion of gut microbiota facilitates the effect of FGF21 on protecting acute pancreatitis in diabetic mice” (Manuscript NO.: 87685, Basic Study) and for providing helpful comments to guide our revision. We have substantially revised the manuscript after reading the comments. When the text was changed, it is highlighted with yellow color in the revised version for easier tracking.

Please find the following detailed responses to your comments and suggestions.

### **Answers to reviewers:**

Reviewer #1: The rat caerulein acute pancreatitis model has a low incidence of pancreatic necrosis and produces relatively mild pancreatitis. Clinically, overuse of antibiotics in acute pancreatitis has been shown to lead to the development of resistant bacteria and is not beneficial. It is necessary to explain in detail the significance of adding antibiotics in the treatment of acute pancreatitis in this study.

### **Answer:**

We would thank the reviewer for raising this question. The current universal mouse AP model is induced by ceruletide, more specifically by seven intraperitoneal injections of ceruletide (50 µg/kg) with a 1-h interval. (1. Peng C, et al. Cell Death Dis. 2023;14(2):155. doi: 10.1038/s41419-023-05655-w. 2. Zhang L, et al. EBioMedicine. 2022; 78: 103959. doi: 10.1016/j.ebiom.2022.103959. 3. Xia D, et al. Free Radic Biol Med. 2020; 147: 139-149. doi: 10.1016/j.freeradbiomed.2019.11.034.

4. Zhao Q, et al. *Gastroenterology*. 2018; 154(6): 1822-1835.e2. doi: 10.1053/j.gastro.2018.01.065. 5. Biczó G, et al. *Gastroenterology*. 2018; 154(3): 689-703. doi: 10.1053/j.gastro.2017.10.012.) Regarding the question of overuse of antibiotics in acute pancreatitis, we have not found any relevant studies about antibiotic abuse in pancreatitis, so we cannot provide a conclusion at the moment.

Reviewer #2:

1) Article title (and results description as a whole). Article title does not quite correctly reflect the study design and findings. According to the manuscript, authors used FGF21 and Abx in a prophylactic mode (before the induction of pancreatitis). Therefore, FGF21 and depletion of gut microbiota PREVENT (or decrease sensitivity to) rather than alleviate ceruletide-induced AP in diabetic mice. Authors should correct the interpretation of obtained results: it was prevention rather than treatment.

**Answer:**

We appreciate it very much for the reminder. Our original writing did indeed cause ambiguity, but now we have corrected the expression of alleviation to prevention, including the article title and text.

2) Introduction. The phenomenon of 'a substantial upregulation of FGF21 in AP' revealed by the authors in preliminary research (Have the results of this research been published?) contradicts with commonly accepted hypothesis considering pancreatitis as an FGF21-deficient state. Please, comment the contradiction.

**Answer:**

Thank you for your feedback. Our preliminary research which was published in Journal of Cellular and Molecular Medicine 2020 underscored a substantial upregulation of FGF21 in AP. Exogenous administration of FGF21 curtails pancreatic injury, aberrant expression of digestive enzymes, and inflammatory response, thus impeding the occurrence of AP. (Chen Q, et al. J Cell Mol Med. 2020;24:5341-5351. doi: 10.1111/jcmm.15190.) Some other studies also showed that expression of FGF21 increased during the development of AP. (1. Luo Y, et al. Cytokine Growth Factor Rev. 2017;38:59-65. doi: 10.1016/j.cytogfr.2017.08.001. 2. Huang Z, et al. Curr Hypertens Rep. 2017;19(4):28. doi: 10.1007/s11906-017-0730-5. 3. Singhal G, et al. PLoS ONE. 2016;11(2):e0148252. doi: 10.1371/journal.pone.0148252.)

3) Methods. Authors should provide citation in the AP model description, and separate section 'Study design' is strongly recommended in order to clarify animal groups description. E.g., what is normal control group (NC)? Are these healthy wild-type mice or db/db mice without AP? Which strain of mice was actually used as a normal control: C57BLKS/J, db/m, or some other? db/db mice without AP cannot be referred as normal control at least because these animals have dysbiosis (decreased Firmicutes abundance, lactobacilli and bifidobacteria count etc). If there was no normal control, please compare groups of manipulated animals (AP group, FGF21-group and FGF21+Abx-group) with db/db animals, and don't refer db/db mice as normal control.

**Answer:**

We appreciate it very much for this good suggestion. The current universal mouse AP

model is induced by ceruletide, and to increase reliability we have added citation in the AP model description. In the current study, all animals are diabetic mice (*db/db*) with fasting blood glucose levels >16.7 mmol/L, and *db/db* mice without AP were used as controls. In order to clarify animal groups, we have modified this expression of NC group to *db* group throughout the text according to the comment. Hope the current expression no longer causes ambiguity.

4) Figures. Figure 2 duplicates data from Figure 3. It is appropriate to use only Figure 3.

**Answers:**

Figure 2 shows that FGF21 treatment improves the composition of gut microbiota, while Figure 3 shows that combined therapy of Abx cocktail and FGF21 alleviates AP in diabetic mice, including diminishing the levels of serum amylase and inflammatory factors, and the minimal damage observed in pancreatic and intestinal tissue sections. These two figures shows different data, so we made two figures to display the data.

We sincerely hope that this revised manuscript has addressed all your comments and suggestions. We appreciated for reviewers' warm work earnestly, and hope that the correction will meet with approval. Once again, thank you very much for your comments and suggestions. We hope the revised manuscript could be acceptable for publication in "World Journal of Diabetes". Thank you in advance for your time and effort!

Best regards,

Sincerely yours,

Fanghua Gong

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2023-09-21

## Round 2

Dear editor,

We sincerely thank the reviewers for thoroughly examining our manuscript entitled “Depletion of gut microbiota facilitates the effect of FGF21 on protecting acute pancreatitis in diabetic mice” (Manuscript NO.: 87685, Basic Study) and for providing helpful comments to guide our revision. We have substantially revised the manuscript after reading the comments. When the text was changed, it is highlighted with yellow color in the revised version for easier tracking.

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### **Answers to reviewers:**

Reviewer:

Please explain the mechanism by which the effect of FGF21 is enhanced by the addition of antibiotics in this study. Also please consider the disadvantages of disrupting the intestinal flora with antibiotics.

### **Answer:**

We sincerely thank you for thoroughly examining our manuscript and for providing

helpful comments to guide our revision. 1) When the mice were treated with a cocktail of antibiotics (Abx), Abx could help eliminate harmful bacteria and play an auxiliary role in the protective effect of FGF21. 2) As for the disadvantages of disrupting the intestinal flora with antibiotics, Abx will damage the intestine. But FGF21 was reported to have the function of repairing intestinal damage, effectively avoiding the damage of Abx treatment to the intestine. (Page17, Line21-Page18, Line13)

We sincerely hope that this revised manuscript has addressed all your comments and suggestions. We appreciated for reviewers' warm work earnestly, and hope that the correction will meet with approval. Once again, thank you very much for your comments and suggestions. We hope the revised manuscript could be acceptable for publication in "World Journal of Diabetes". Thank you in advance for your time and effort!

Best regards,

Sincerely yours,

Fanghua Gong

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2023-10-12