Dear Editor and Reviewers:

Thank you for your letter and the reviewers' comments on our manuscript titled "Enhanced Glucose Homeostasis via Clostridium *symbiosum*-Mediated GLP-1 Inhibition of Hepatic Gluconeogenesis in Mid-Intestinal Bypass Surgery" (Manuscript No.: 86896, Basic Study). These comments were very helpful for revising and improving our paper and have important guiding significance for other research. We have studied the comments carefully and made corrections that we hope will be met with approval. The main corrections are in the manuscript, and the responses to the reviewers' comments are as follows (the replies are highlighted in blue).

Replies to the reviewers' comments:

Reviewer #1:

1. **Specific Comments to Authors: The author reported the postoperative intestinal bypass of the midsection small intestine in streptozotocin-induced diabetic rats improves glucose metabolism by increasing GLP-1 levels and inhibiting hepatic gluconeogenesis through the increased abundance of intestinal *Clostridium_symbiosum*.**

Response:

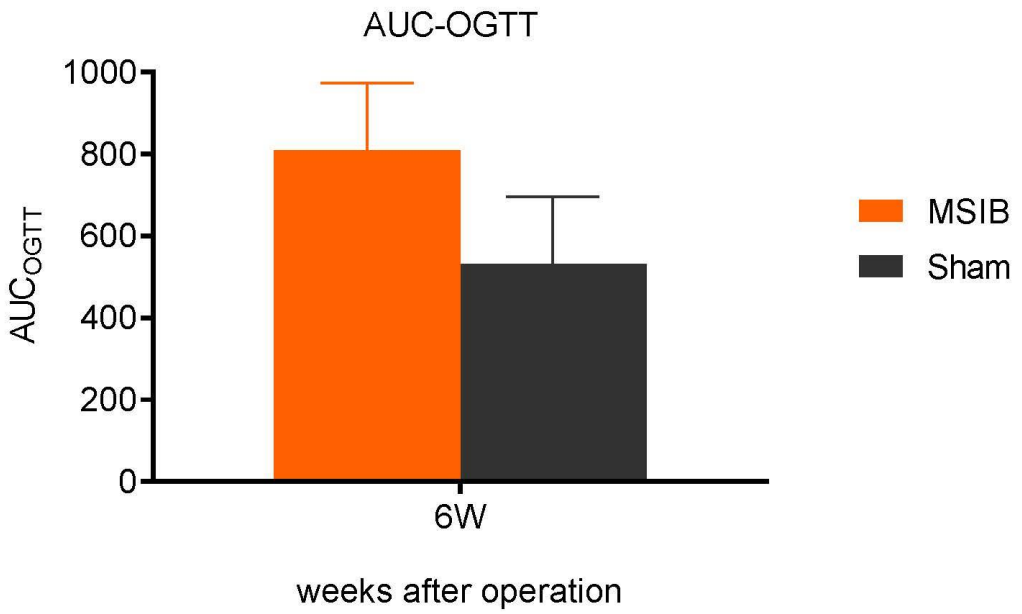
Thank you for your recognition of our research. As you mentioned, we have explored some of the mechanisms behind the improvement in glucose metabolism following mid-small intestinal bypass surgery through our experiments. We will continue to delve deeper into the relationship between the small intestine and glucose metabolism. Once again, we appreciate your acknowledgment.

Reviewer #2:

1. **Methods Why did they use 1 g/kg of glucose in the OGTT? The most used dose of glucose is 2 g/kg. A bibliographical reference is needed.**

Response:

Thank you for your comment. As you rightly pointed out, the standard dose for an OGTT is 2 g/kg[1]. However, in our previous experiments, we found that using this dose of glucose in STZ-induced SD rats led to a rapid increase in blood glucose levels, exceeding the upper limit of the glucose meter at 33.4 mmol/L. This made it difficult to accurately observe changes in blood glucose concentrations. Furthermore, our investigations revealed that the OGTT curves for the experimental group and the control group differed only in baseline blood glucose levels. The overall curve shapes were similar, and when we compared the area under the curve (AUC) minus the baseline area for the OGTT results at week 6, there was no significant difference between the two groups (as shown in the figure below, $P > 0.05$). This finding suggests that there was no significant change in insulin secretion or insulin sensitivity in the short term after mid-small bowel bypass surgery. Therefore, we opted to use a dose of 1 g/kg to assess changes in glucose metabolism between the experimental and control groups of rats.



2. **They need to specify the number of animals per group and how did they calculate it?**

Response:

Thank you for your comment. In determining the number of rats in each group, we consulted a statistician who evaluated the sample size needed to detect differences in glucose metabolism. The estimation of sample size was based on changes in blood glucose levels in diabetic rats and was calculated using the following formula:

Formulas

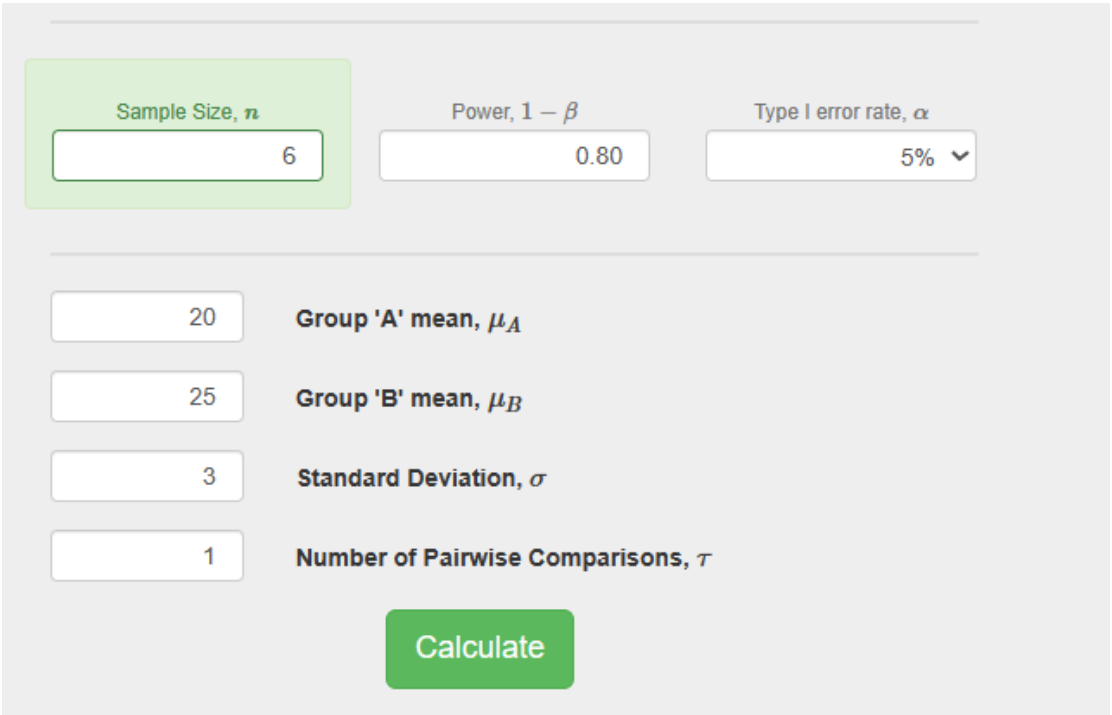
This calculator uses the following formulas to compute sample size and power, respectively:

$$n = 2\left(\sigma\frac{z_{1-\alpha/(2\tau)} + z_{1-\beta}}{\mu_A - \mu_B}\right)^2$$

$$1 - \beta = \Phi\left(z - z_{1-\alpha/(2\tau)}\right) + \Phi\left(-z - z_{1-\alpha/(2\tau)}\right) \quad , \quad z = \frac{\mu_A - \mu_B}{\sigma\sqrt{\frac{2}{n}}}$$

where

n is sample size
σ is standard deviation
Φ is the standard Normal distribution function
Φ^{-1} is the standard Normal quantile function
α is Type I error
τ is the number of comparisons to be made
β is Type II error, meaning $1 - \beta$ is power



In the end, we calculated a sample size of 6 rats per group. In fact, one rat in the experimental group succumbed to postoperative abdominal infection,

resulting in a final inclusion of 7 rats in the experimental group and 6 rats in the control group.

3. **First line of the section 3 "Biochemical tests and ELISA" It´s not clear.**

Response:

Thank you for your keen observation. We have made the necessary corrections in the original text and highlighted them in yellow. The revised version is as follows:

Blood was collected from the rat tail vein, and the blood glucose level was measured using an electronic glucometer (Accu-Chek Performa, Roche Diagnostics, Switzerland).

4. **Did you collect blood samples in a glucometer? Or did you use glucometer to measure blood glucose levels?**

Response:

Thank you for your comment. In our experiment, we directly measured the blood glucose concentration in rat tail vein blood using an electronic glucometer and did not collect blood for glucose measurement.

5. **What kind of insulin were used in the experiment? Isophane? rapid insulin? Bibliographical reference is needed.**

Response:

Thank you for your comment. The insulin we used was human insulin (Wanbang Biopharmaceuticals, Jiangsu, China), which is a short-acting insulin. This information has been provided in the manuscript along with the relevant references[2, 3].

6. **Metabolomics section, change "faeces" for "feces".**

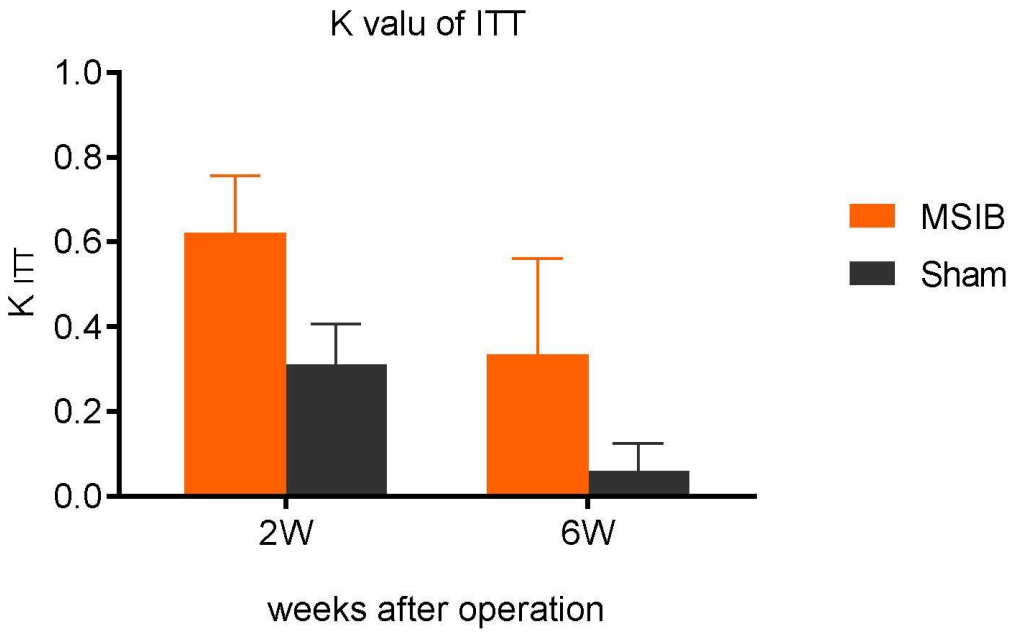
Response:

Thank you for the feedback. We have corrected the wording in the manuscript

accordingly.

7. For Analysis of ITT they have to calculate KITT for 2-W and 6-W.

Response: Thank you for your comment. Below is the analysis graph for KITT. As shown in the figure, there was no significant difference in the rate of glucose reduction in the ITT tests between the experimental group and the control group at 2 weeks and 6 weeks post-surgery.



8. How did they measure glycogen content?

Response:

Thank you for your question. To compare the hepatic glycogen content, we utilized the positive cell area percentage analysis method: Three 200× fields of view were selected and photographed from each tissue slice. During photography, efforts were made to ensure that the tissue filled the entire field of view, ensuring consistent background lighting for each photo. Image-Pro Plus 6.0 software (Media Cybernetics, Inc., Rockville, MD, USA) was used to select the same shade of purple as the uniform criterion for judging positivity in all photos. Each photo was then analyzed to determine the area of positive cell pixels in relation to the total tissue pixel area, yielding the percentage of

positive area (%).

9. Injection only of STZ generates a hyperglycemic animals, but this hyperglycemia is more associated to type 1 than type 2 diabetes, in further experiments STZ-NA model is better to test type 2 diabetes.

Response:

Thank you for your suggestion. As you rightly pointed out, the STZ-induced diabetic rat model closely resembles type 1 diabetes, initially characterized by an absolute lack of insulin. However, over time, these rats may also exhibit symptoms of insulin resistance. In our future studies, we will consider other models to investigate the impact and mechanisms of mid-small intestinal bypass surgery on type 2 diabetes.

10. Discussion Gluconeogenesis inhibition explains the reduction of FBG. What is the hypothesis in the reduction of OGTT?

Thank you for your comment. The decline in OGTT primarily reflects differences in the baseline blood glucose levels between the two groups of rats. As mentioned in question 1, when we subtracted the baseline blood glucose from the area under the OGTT curve for both groups of rats, there was no statistically significant difference. This is consistent with the results of the ITT, indicating that insulin sensitivity or insulin secretion in the experimental group of rats did not show a significant improvement.

1 Virtue S, Vidal-Puig A. GTTs and ITTs in mice: simple tests, complex answers. *Nature metabolism* 2021; **3**(7): 883-886 [PMID: 34117483 DOI: 10.1038/s42255-021-00414-7]

2 Paudel YN, Ali MR, Shah S, Adil M, Akhtar MS, Wadhwa R, Bawa S, Sharma M. 2-[(4-Chlorobenzyl) amino]-4-methyl-1,3-thiazole-5-carboxylic acid exhibits antidiabetic potential and raises insulin sensitivity via amelioration of

oxidative enzymes and inflammatory cytokines in streptozotocin-induced diabetic rats. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie* 2017; **89**: 651-659 [PMID: 28262618 DOI: 10.1016/j.biopha.2017.02.043]

3 Liu Y, Fu X, Chen Z, Luo T, Zhu C, Ji Y, Bian Z. The Protective Effects of Sulforaphane on High-Fat Diet-Induced Obesity in Mice Through Browning of White Fat. *Frontiers in pharmacology* 2021; **12**: 665894 [PMID: 33995092 PMCID: PMC8116735 DOI: 10.3389/fphar.2021.665894]