**Responses to the Reviewers’ and Editor’s Comments**

Manuscript NO.: 69463

Manuscript title: " The uses of knockout, knockdown, and transgenic models in the studies of glucose transporter 4"

The authors would like to thank the reviewers and editor for the time and efforts, especially those excellent comments. We have revised the manuscript according to the reviewer’s comments. Here are our responses to the reviewers and editor’s comments line-by-line. The comments are shown first, which are followed by our responses in italics.

Please note that the words and sentences that have been revised are highlighted in yellow. In addition, we have deleted and added references. Therefore, the reference list can be considered revised.

1. Reviewer #1.

1.1 As mentioned, CRISPR has been developed and used widely, GLUT4 knockout/knockdown through this system may be worth to be done. However, you did not compare the variation of the indicated method with CRISPR. Why?

*Responses: We agreed with the reviewer that CRISPR has become a very popular technique to study functions of a gene. We have used “GLUT4” and “CRISPR”, and “SLC2A4” and “CRISPR” as key words to search PubMed, and retrieved 8 and 3 published articles, respectively. However, none of the published articles used CRISPR to knockout GLUT4 gene in cells or animals. A similar search in GOOLGE retrieved articles that used CRISPR to study the functions of proteins involved in exocytosis of GLUT4 translocation mechanisms, which is beyond the scope of our paper. However, none of those studies used CRISPR method to delete GLUT4 gene. We were not sure why this is the case. Therefore, it is not possible to conduct the comparison as suggested by the reviewer. We have added several sentences in the second to the last paragraph of section 4 (Conclusion section) to indicate that. It is a good project to do in the future.*

1.2. This submission seems going to introduce the old methods regarding transgenic overexpression and tissue specific knockout of GLUT4 gene. Did you have the experience on it? Publication of the used transgenic mice is helpful.

*Responses: We have studied the glucose and glycogen metabolism in response to the stimulation of insulin and retinoic acid (RA) in L6 skeletal muscle cells. We found that RA synergized with insulin to increase glucose usage in L6 cells during differentiation (Goff, M. and Chen G. Long-Term Treatment with Insulin and Retinoic Acid Increased Glucose Utilization in L6 Muscle Cells via Glycogenesis. Biochem Cell Biol. 2020 Dec;98(6):683-697. doi: 10.1139/bcb-2020-0131). When we tried to study the contribution of GLUT4 system, we encountered a problem and could not find an antibody to give us reliable results of GLUT4 expression. This triggered us to summarize all the GLUT4 studies. Based on the summarized studies presented here, only traditional methods had been used to overexpress or knockout of GLUT4 gene to study its functions. Those are the only results that we have found after search in PubMed. It appears that GLUT4 gene has not been knockout or knockdown or overexpressed recently in cells or transgenic animals using more recent techniques such as CRISPR as we elaborated our response to your first comment (item 1.1). We believe that is the status of the GLUT4 field. This could also be a challenge for those who are trying to confirm their knockdown, knockout or transgenic results using more recent molecular biology tools such as CRISPR. Since this is a review paper, we do not have transgenic results to publish. In addition, we do not have those transgenic animals in our lab. Hopefully, we can create some tools to study GLUT4 functions in the future.*

1.3. The fasting state has been indicated in conclusion without evidence.

*Response: Thank you for pointing this out. We have added a reference. Please see the conclusion section for the change.*

1.4. This one seems a report for Yantai Zestern Biotechnique Co. Ltd. Formation was far from the scientific version.

*Response: In the acknowledge of this manuscript, we thanked Yantai Zestern Biotechnique Co. Ltd for its support to our research program, which was to use their Quantitative Dot Blot technology to study the absolute protein expression. Out submitted review manuscript is not a report for this company. The reason that we conducted this summary work has been stated in the responses to the previous two comments.*

1.5. Merits and limitations were ignored in this report.

*Response: Thank you for your comment. We have added a couple of sentences in the first paragraphs and second to the last paragraph of section 4 (Conclusion section) to state the merits and limitations, respectively. Please see the revised manuscript for the changes.*

2. Reviewer #2:

2.1 The topic is interesting, but I think the manuscript can be accepted for publication after some revisions. 1. Please revise the references carefully to agree with the format of the journal.

*Responses: Thank you for pointing this out. We have re-formatted the reference style. Please see the resubmitted manuscript for the changes.*

2.2. Some names should be italized, all were marked in the text.

*Responses: Thank you for pointing it out. We have checked the manuscript and italicized the E. coli, and gene names accordingly. Please see the revised manuscript for the changes.*

2.3. Other revisions were marked in the text.

*Responses: We have marked the revisions.*

3. Editorial Office's comments

3.1 The topic is of interest. However, a comprehensive analysis of more recent and relevant contributions in this field is lacking. In fact, only 10% of cited references represent publications from the last 5 years.

*Responses: We agreed with the editor. GLUT4 has been considered a key player in the insulin—stimulated glucose uptakes in the muscle and adipose tissues. However, the studies using more recently developed molecular biology tools such as CRISPR in cells and animals have not been done. Therefore, we can only get those relevant studies, which were published more than 5 years ago. Please see our responses to reviewer #1 for our reasoning behind this phenomenon. On the other hand, it also indicates that more studies should be done in this line of research. Regarding to the more recently references, we have revised the reference list and used more recently publications, which should have increased the percentage of references published in the last 5 years. Please see the revised manuscript for the changes.*

3.2 Furthermore, a method section describing how authors selected references is lacking.

*Response: Thank you for pointing this out, we have added several sentences at the end of the Section 1 to elaborate what we had done. In addition, the detailed descriptions of the methods are included in each subsection. Please see revised manuscript for the changes*.

3.3 Finally, the translational value of this review has not been clearly depicted by authors.

*Response: Thank you for pointing this out. We have added several sentences Section 4 (Conclusion section) to reflect this. Our review points out that additional studies using recently developed CRISPR method will benefit the field. In addition, the role of GLUT4 in the fasting state may extend our view of GLUT4’s role in the metabolic homeostasis.*

4. Company Editor-in-Chief: I recommend the manuscript to be published in the World Journal of Meta-Analysis.

*Responses: Thank you for your comments. Our goal is to summarize the current understanding and help the field to move forward. If the World Journal of Diabetes is not suitable for this, the World Journal of Meta-Analysis also works. Thank you for your consideration.*