Manuscript ID: 72816
Title: Functional annotation and enrichment analysis of differentially expressed proteins in serum of patients with type 2 diabetes mellitus after dapagliflozin treatments
Journal: World Journal of Diabetes

Response to Reviewers' comments

Dear Editor,

We thank you for your careful consideration of our manuscript. We appreciate your response and overall positive initial feedback and made modifications to improve the manuscript. After carefully reviewing the comments made by the Reviewers, we have modified the manuscript to improve the presentation of our results and their discussion, therefore providing a complete context for the research that may be of interest to your readers.

We hope that you will find the revised paper suitable for publication, and we look forward to contributing to your journal. Please do not hesitate to contact us with other questions or concerns regarding the manuscript.

Best regards,
Zhao-Li Yan

Reviewer #1

1. How was the number of subjects included in the study determined? Power test and sample size calculation should be done.

Response: We thank the Reviewer. It was an exploratory study that used convenience sampling. The aim of this study was mainly to identify differentially expressed serum proteins after dapagliflozin treatment. It was noted in the Limitations.

2. Baseline values of the control group and compare results with the study group should be given.

Response: We thank the Reviewer. We provide a new table (now Table 1) that compared the baseline characteristics of the two groups.

3. Patients aged 25 years and older were included in the study. Type 2 diabetes usually occurs in people aged 35 and over. Were other types of diabetes exclude in the patients?

Response: We thank the Reviewer. We agree that the risk of T2DM becomes more significant starting at 35 years of age, but with the increasing epidemics of childhood obesity, it is expected to see T2DM in younger patients. In this study, there were three patients younger than 35 years in each group (26, 30, and 31 years in the control group; 33, 34, and 34 years in the dapagliflozin group). These had been diagnosed with T2DM, and other types of non-type 2 DM have been excluded.

4. Authors gave only exercise and diet to the control group. The mean HbA1c values of the patients included in the study were 8. Is this ethically correct behavior?

Response: We thank the Reviewer for the comment. We did not instruct these patients to be treated only using diet and exercise; it was their own choice. Still, we follow such patients to be able to start drug therapy if diet and exercise are no longer able to control their T2DM. They were enrolled as controls, but we did not intervene on them. Therefore, it is ethical. It was clarified in the Methods – Patients.

All participants received diet and exercise guidance. The dietary guidance referred to the balanced dietary plan recommended by the Chinese Guidelines for the Prevention and Treatment of Type 2 Diabetes (2013 edition) and suggested 1/3 structure of the energy intake ratio of three meals or 2:2:1 distribution. According to the "Guidelines for Exercise in Type 2 Diabetes Mellitus", the exercise program was mainly composed of low-intensity aerobic exercise such as walking, swimming, cycling, etc. The participants were required to exercise after a meal for about 30 min and 3-5 times per week. No adverse reactions such as urinary tract infection, ketoacidosis, and other adverse reactions occurred during the study.

The average value of HbA1c was 8%. Specifically, before dapagliflozin was administered, the condition for inclusion was that the HbA1c was <7% after treatment, so the average value after the medication was 6.48%. HbA1c in the control group was 6.37% (new Table 1).

5. The number of patients and controls included in the study is less than 30. Therefore, nonparametric tests such as Wilcoxon, spearman, and Mann-Whitney u should be used.

Response: We thank the Reviewer. Please see the new Tables 1 and 2.

6. Table 2 has no contribution to the study.

Response: It was deleted.

Reviewer #2

1. It is known that pyruvate carboxylase is a mitochondrial protein, and integrins are transmembrane receptors located on the cell membranes. The sources of these proteins in the patients' serum need to be discussed.

Response: We thank the Reviewer for the comments. First, a major misunderstanding arose from an error that occurred during manuscript translation and preparation: PCX stands for podocalyxin, which is the major surface antigen of podocytes, not for pyruvate carboxylase (which is abbreviated PCx). PCX is the main surface antigen of podocytes and is normally expressed in renal podocytes, endothelial cells, and vascular endothelial cells and participates in maintaining the vascular endothelial cell barrier and reducing vascular inflammation. High glucose levels downregulate the expression of PCX in cultured podocytes via ERK1/2 MAPKs and inhibit the expression of PCX protein and mRNA by WT1 tumor protein and advanced glycation end-products[1], possibly resulting in the reduction of PCX in the blood. Second, we agree that integrins are cellular proteins that would not be expected to be found in circulation. Still, this study was not designed to determine the source of these proteins in the plasma. On the other hand, numerous studies report serum/plasma levels of various integrins as markers of diseases[2-4]. It could be hypothesized that the systemic inflammatory condition observed in T2DM increases cell death, releasing those proteins in circulation, but the present study cannot provide an answer regarding that point. Future studies will have to examine that specifically. We nevertheless added a statement in the Discussion.

2. The possible effect of dapagliflozin treatment on mitochondria needs to be discussed in more detail. Some studies showed that T2DM is associated with the development of mitochondrial dysfunction in vital tissues and organs (DOI: 10.3390/ijms21186559), and the treatment with dapagliflozin may restore the ultrastructure and functions of mitochondria in experimental T2DM (DOI: 10.1016/j.mito.2021.06.008).

Response: We thank the Reviewer. We added some discussion about that.

2. It is necessary to provide the catalog numbers of the BioRad kits used for the determination of MPO, alpha II beta integrin, and PCX proteins by enzyme-linked immunosorbent assay.

Response: The catalog numbers were CSB-EL0118644HU for integrin, CSB-E08721h for MPO, and CSB-E09891h for PCX. They were added to the manuscript.

Editorial comments

5 ABBREVIATIONS

In general, do not use non-standard abbreviations, unless they appear at least two times in the text preceding the first usage/definition. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, and mAb, do not need to be defined and can be used directly.

The basic rules on abbreviations are provided here:

(1) Title: *Abbreviations are not permitted. Please spell out any abbreviation in the title.*

Response: There were no abbreviations in the title.

(2) Running title: *Abbreviations are permitted. Also, please shorten the running title to no more than 6 words.*

Response: The running title now has five words.

(3) Abstract: *Abbreviations must be defined upon first appearance in the Abstract. Example 1: Hepatocellular carcinoma (HCC). Example 2:* Helicobacter pylori *(*H. pylori*).*

Response: It was revised.

(4) Key Words: *Abbreviations must be defined upon first appearance in the Key Words.*

Response: It was revised.

(5) Core Tip: *Abbreviations must be defined upon first appearance in the Core Tip. Example 1: Hepatocellular carcinoma (HCC). Example 2:* Helicobacter pylori *(*H. pylori*)*

Response: It was revised.

(6) Main Text: Abbreviations must be defined upon first appearance in the Main Text. Example 1: Hepatocellular carcinoma (HCC). Example 2: *Helicobacter pylori* (*H. pylori*)

Response: It was revised.

(7) ***Article Highlights:*** *Abbreviations must be defined upon first appearance in the Article Highlights. Example 1: Hepatocellular carcinoma (HCC).*

Example 2: Helicobacter pylori *(*H. pylori*)*

Response: It was revised.

(8) Figures: *Abbreviations are not allowed in the Figure title. For the Figure Legend text, abbreviations are allowed but must be defined upon first appearance in the text. Example 1: A: Hepatocellular carcinoma (HCC) biopsy sample; B: HCC-adjacent*

tissue sample. For any abbreviation that appears in the Figure itself but is not included in the Figure Legend textual description, it will be defined (separated by semicolons) at the end of the figure legend. Example 2: BMI: Body mass index; US: Ultrasound.

Response: It was revised.

(9) Tables: *Abbreviations are not allowed in the Table title. For the Table itself, please verify all abbreviations used in tables are defined (separated by semicolons) directly underneath the table. Example 1: BMI: Body mass index; US: Ultrasound.*

Response: It was revised.

Science editor

In this study, the roles ofαIIβ integrin, MPO and PCX in the regulation of multiple pathways were discussed in detail. And found that dapagliflozin has hypoglycemic effects, and regulates the serum expressions of MPO, ITGA2B and PCX, possibly contributing to the effects of dapagliflozin on oxidative stress, insulin resistance and lipid metabolism. This is an interesting study.
Language Quality: Grade B (Minor language polishing)
Scientific Quality: Grade B (Very good)

Response: We thank the Science Editor for the comments. The manuscript was proofread.

Company editor-in-chief

I have reviewed the Peer-Review Report, the full text of the manuscript, and the relevant ethics documents, all of which have met the basic publishing requirements of the World Journal of Diabetes, and the manuscript is conditionally accepted. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report, Editorial Office's comments and the Criteria for Manuscript Revision by Authors. Please provide decomposable Figures (in which all components are movable and editable), organize them into a single PowerPoint file. Please authors are required to provide standard three-line tables, that is, only the top line, bottom line, and column line are displayed, while other table lines are hidden. The contents of each cell in the table should conform to the editing specifications, and the lines of each row or column of the table should be aligned. Do not use carriage returns or spaces to replace lines or vertical lines and do not segment cell content. The title of the manuscript is too long and must be shortened to meet the requirement of the journal (Title: The title should be no more than 18 words).

Response: We thank the Editor-in-Chief for the comments. All figures are now provided in PowerPoint. All tables were revised as three-line tables. All lines and columns are aligned. The title is now no longer than 18 words.

References

1 Drossopoulou GI, Tsotakos NE, Tsilibary EC. Impaired transcription factor interplay in addition to advanced glycation end products suppress podocalyxin expression in high glucose-treated human podocytes. Am J Physiol Renal Physiol 2009; 297: F594-603 [PMID: 19605546 DOI: 10.1152/ajprenal.00203.2009]

2 Yamauchi M, Mizuhara Y, Maezawa Y, Toda G. Serum levels of integrins in chronic liver diseases. Pathol Res Pract 1994; 190: 984-992 [PMID: 7534915 DOI: 10.1016/S0344-0338(11)81005-8]

3 Radwan AF, Ismael OE, Fawzy A, El-Mesallamy HO. Evaluation of Serum Integrin alphavbeta3 & Vitronectin in the Early Diagnosis of Breast Cancer. Clin Lab 2019; 65 [PMID: 31307158 DOI: 10.7754/Clin.Lab.2019.181219]

4 Lenggenhager D, Bengs S, Fritsch R, Hussung S, Busenhart P, Endhardt K, Topfer A, The FO, Butikofer S, Gubler C, Scharl M, Morell B. beta6-Integrin Serves as a Potential Serum Marker for Diagnosis and Prognosis of Pancreatic Adenocarcinoma. Clin Transl Gastroenterol 2021; 12: e00395 [PMID: 34388137 PMCID: PMC8367066 DOI: 10.14309/ctg.0000000000000395]