Author’s response to reviews

Title: Increment of plasma glucose by exogenous glucagon is associated with present and future renal function in type 2 diabetes a retrospective study from glucagon stimulation test.

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Point-by-Point Response to Reviewers

Reviewer 1:

Criticism 1

(Abstract) "since we hypothesized that Δglucose is associated with the liver and renal function reflecting the capacity for gluconeogenesis in the organs." The reviewer would like to know whether the liver and renal function could reflect the capacity for gluconeogenesis in the organs. Please show the data or cite previous studies in the Background section.

Response to Criticism 1
We really appreciate the reviewer’s comment. We cited previous studies concerning gluconeogenesis in pathophysiological conditions in kidney and liver diseases (Background section, page 3-4).

Criticism 2

(Methods) Please describe how to obtain informed consent from subjects.

Response to Criticism 2

We thank for the reviewer’s comment. Participants in the current study were informed through an opt-out consent form which stated that previously obtained medical information would be used in this retrospective study on the Asahikawa Medical University Research Ethics Committee’s web site.

Criticism 3

(Discussion) "Indeed, liver and kidney are almost equivalently involved in glucose production via gluconeogenesis in the post-absorptive state of normal subjects." This sentence seems like a contradiction, since GST was carried out at 8 am after an overnight fast in this study.

Response to Criticism 3

We thank the reviewer’s comment. However, we believe that our description was not inappropriate because the ‘post-absorptive state’ generally refers to the time period when energy is obtained by glycogenolysis or gluconeogenesis, such as during sleep or after an overnight fast (Gerich JE. Diabet Med. 2010; 27: 136-142.). So, it is commonly accepted that ‘post-absorptive state’ is almost similar to ‘fasting state’, in contrast to the antonym of ‘absorptive state’.

Criticism 4

(Discussion) "Previous several studies already showed that obesity, glycemic control, serum lipids, and proteinuria were respectively the predictors for the development of chronic kidney disease." In this study, BMI and HbA1c were positively associated with eGFR one year later, which seems to be the opposite result of the previous study (Yamagata K, et al. Kidney Int. 2007; 71: 159-166). Please explain.

Response to Criticism 4

We thank for the reviewer’s insightful comments. As the reviewer’s point, the relationship between future renal function and both BMI and glycemic status in the current study was opposite to the previous study conducted by Yamagata and colleagues (Yamagata K, et al. Kidney Int. 2007; 71: 159-166). Our current study was conducted with only patients with type 2
diabetes who were under treatment, while the previous study was conducted with general population including subjects without diabetes. We consider that such difference in study population might have led to the different results.

Point-by-Point Response to Reviewers

Reviewer 2:

Comment 1

Renal function/impairment is clearly related to eGFR, creatinine and cystatin C however the "liver function" tests included are less clearly measures of actual liver function so this makes it harder to test for a relationship between liver function and glucose increment. In addition, there was a wide eGFR range but seemingly a narrower ALT, AST, INR and albumin range. There is brief mention of this in the discussion but it should be more specifically addressed as a limitation of the study. It ultimately makes it hard to dissect out the respective roles of the liver and kidney in gluconeogenesis and to fully address the hypothesis.

Response to Comment 1

We really appreciate the reviewer’s important point. According to the suggestion, we added descriptions concerning the limitation of using ALT, AST, PT and albumin for assessing liver function and residual liver function in discussion, since subjects even with moderate liver dysfunction were not included in our study (Discussion section, last paragraph on page 9 and the middle of first paragraph on page 10).

Comment 2

The patients with diabetes had a high mean HbA1c. This may reflect attendees. Is there any way to comment on the results for those with better controlled diabetes or does this require a further study?

Response to Comment 2

We thank for the reviewer’s perspective comment. As the reviewer’s point, our results might be affected by the characteristics of the subjects with high HbA1c levels, although we conducted glucagon stimulation test after adequate glycemic control. This is one of the limitations of this study, so we mentioned about that in Discussion (Discussion section, 3rd paragraph on page 11).

Comment 3

Please clarify insulin therapy prior to the test. It is stated that short acting insulins were not give. What specifically was done about any long acting insulins.
Response to Comment 3

We thank the reviewer’s comment. Some patients received long acting insulin such as insulin glargine, insulin detemir and insulin degludec subcutaneously at 8 pm the day before GST to maintain adequate fasting blood glucose levels. We now added this description in Methods (Methods section, 2nd paragraph on page 5).

Comment 4

Figures 1a, 1B and 1C don't correlate well with the text

Response to Comment 4

We really appreciate the reviewer’s indication. We corrected the correlation between the text and figure 1A, B, and C (Results section, 2nd paragraph and 3rd paragraph on page 7).