Manuscript ID 60176 entitled " Unaffected blood glucose after oral lactulose intake in type 2 diabetic individuals"

POINT-BY-POINT REPLY TO THE REVIEWERS' COMMENTS

The authors would like to thank the reviewers for their valuable criticisms which have been taken into full consideration in the revised version of the manuscript and which definitely contributed to the improvement in the quality of their paper.

Authors' reply to reviewer #1:

• I found both the title "Unaffected blood glucose after oral lactulose intake in type 2 diabetic individuals" and the statement in the abstract • "all secondary endpoints, including the maximum increase in glucose from baseline, did not differ between lactulose and water intake in a clinically relevant manner" somewhat exaggerated and overstated. There is no such thing in medical science that "unaffected blood glucose" and this clearly indicates the problem with the paper:

• If the authors would have had used the appropriate language, e.g. "unchanged blood glucose levels" in the title it would not be true any more. It is simply due to that a significant difference (p=0.0059) was observed in the maximum increase in glucose from baseline after the administration of 30g of liquid lactulose vs administration of water.

• Therefore I would suggest to change the title to a more relevant one.

In line with the reviewers' request, we have modified the title of our manuscript. The new title is "*Blood glucose response after oral lactulose intake in type 2 diabetic individuals*".

• Similarly, it is not entirely appropriate to state that "all secondary endpoints, including the maximum increase in glucose from baseline, did not differ between lactulose and water intake in a clinically relevant manner" especially not in the results section.

• It should rather be indicated that there was a significant difference in the maximum increase in glucose from baseline (Treatment Diff in max increase of glucose cc= 0.63mmol/L, p=0.0059).

Throughout the manuscript, we have consistently replaced the statement that *"all secondary endpoints, including the maximum increase in glucose from baseline, did not differ between lactulose and water intake in a clinically relevant manner".* Instead, it is now clearly stated that a small, significant increase in maximum blood glucose was observed for liquid lactulose at a dose of 30 g compared to water, in the abstract (page 5), in the results section (page 13), and in the discussion (pages 17).

• In the conclusion it may be stated that this significant difference is not too high, therefore it is unlikely to cause major changes in the clinics, however it is again not entirely black and white and not absolutely equivalent with a kind of "zero clinical relevance" as suggested by the authors with the current interpretation. Even such a small increase may have clinical relevance, it simply depends on the clinical situation, e.g.: a patient with T2DM have a mean postprandial values in his/her SMBG profile of 7.7 mmol/L after a given meal and when he/she starts to load 30g of liquid lactulose this postprandial glucose level increases up to 8.3 mmol/L (as typically patients do not only consume water) and such a postmeal glucose value (already can again be considered above the target) may trigger changes in therapy. Although it really can be recognized that the increase of 0.6 mmol/L in glucose concentration can be considered minimal which - in the very vast majority of the cases - is unlikely to result in major changes of the therapy and this can be outlined in the discussion/conclusion section.

We have modified the conclusion in the abstract (please see page 5) in the full text (please see page 20), and in the research conclusion (page 21). It is now more precisely stated that "blood glucose **$AUC_{baseline\_c\ (0\text{-}180\ min)}$ levels** in mildly constipated, non-insulin dependent subjects with T2DM are not affected by the carbohydrate impurities contained in crystal or liquid lactulose formulations."

Moreover, we have thoroughly revised our discussion on the significance of the observed higher values for C_{max} and maximum increase compared to water with the 30 g dose of liquid lactulose compared to water (please see page 17). We would like to point out that "maximum increase" is a secondary endpoint in our study, solely based on a single sampling point and calculation, not reflecting interindividual variability in blood glucose curves and patterns. Furthermore, individual glucose profiles show a rather heterogenic pattern indicating that this observation presumably was due to random variability. Moreover, we consider this results clinically not relevant since the upper limit of the CI is clearly below the 2.2 mmol/L threshold of clinical relevance and $AUC_{baseline\_c\ (0\text{-}180\ min)}$ was comparable to that of water.

• I would also suggest to report numerically and clearly the results regarding the treatment difference in max increase of glucose cc (0.63mmol/L, p=0.0059) for 30g liquid lactulose vs water in the results section of the abstract already.

We have added a paragraph containing the requested information to the results section of the abstract (please see page 5).

• The clinical interpretation of this should not be mentioned in the results section, but in the conclusion/discussion sections and I would suggest to temper down the tone of this interpretation a bit, in particular for the clinics when liquid lactulose is often used in higher doses (in other indications, e.g.: in PSE high doses can be used up to 300 mL lactulose) and if the currently minimal, yet already significant increases in max increase of glucose levels are multiplied with the use of higher doses (in other indication) than the increases in blood glucose levels can already be clinically more relevant. This is not too obvious now from reading the title at this point.

As requested, we have deleted the sections containing an interpretation of results and moved the respective information to the discussion. Moreover, we have added information on the recommended starter and maintenance doses of lactulose according to the prescribing informations of the lactulose products investigated, which are 30 g and 10-20 g/day, respectively, in patients with chronic constipation (please see discussion, page 17). We are aware that higher lactulose doses may be indicated in other patient settings, such as in portocaval encephalopathy. Yet, these indications were beyond the scope of our investigation which was designed to address potential effects of carbohydrate impurities contained in lactulose when administered at doses recommended for patients with T2DN and chronic constipation in an outpatient setting.

Authors' reply to reviewer #2:

The study adopted numerous exclusion criteria in diabetic patients for the study. It is known that patients with DM have a high frequency of acute and chronic comorbidities and may also receive a high number of medications. This raises the following question: what was the rate of selection of patients after applying all these selection criteria in this study who may receive the benefits of the medication?

Likewise, if the decision of excluding a probably high number of patients could not significantly modify results that might be obtained in the real-life clinical practice. I recommend authors to discuss based

on their results which are finally the contraindications of giving lactulose to diabetic patients in general terms.

The inclusion and exclusion criteria were chosen to reach a homogenous population of individuals with non-insulin-dependent T2DM, without any confounding endocrine or gastrointestinal comorbidity. We have now included the main reasons and numbers for ineligibility during pre-screening failure in the caption to figure 1. These included not interested, missing signs of functional constipation, insufficient blood glucose control, gastrointestinal or other relevant comorbidity (e.g. cancer). Insulin-treated subjects were excluded for safety reasons (blood glucose fluctuations with blood glucose lowering medication applied after 180 min after dosing). Since our aim was to specifically investigate the influence of lactulose on blood sugar response, it had to be ensured that the influence of medications or comorbidities possibly masking any potential effects of lactulose could be ruled out. It is common practice to define strict inclusion / exclusion criteria for clinical studies in order minimize the influence of potential confounders and achieve a certain degree of homogeneity (please see discussion, page 20).

In addition, there are different formulations of lactulose marketed by various laboratories. As a gesture of clarity for readers who want to use lactulose in their diabetic patients in the real-life clinic, what would be the specific recommendations regarding the pharmacological presentation of the lactulose that should be used?

Generally, liquid lactulose is more commonly used in practice. Based on the $AUC_{baseline\_c\ (0\text{-}180\ min)}$, 20 g and 30 g doses of lactulose (crystals or liquid) can be used in mildly constipated, non-insulin dependent subjects with T2DM.

Authors' reply to reviewer #3:

How isomerization process can produce impurities? This information needs to be revised.

In line with the reviewer' request, we have provided information on the generation of impurities in the introduction (please see page 7)

What is still water?

We have modified the term to "non-sparkling water" (please see page 10)

Authors' reply to EDITORIAL OFFICE'S COMMENTS

The figures and tables are now provided as separate ppt and word files, respectively.