Author's response to reviews

Title: Effects of human insulin and insulin aspart preparations on levels of IGF-I, IGFBPs and IGF bioactivity in patients with type 1 diabetes

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Author's response to Reviewer

Thank you for kind letter and pointing out the limitations of our original submission and for guiding the directions for making revisions.

Following your comments, we have revised the manuscript. We hope that our revision has addressed these limitations. The details are as follows (one-by-one, your specific comments is followed by our response):

Editor's Comments:

In addition, I would like to ask if the ANOVA test used for the analysis of a cross-over is the most indicate statistical analysis or if another test, for instance the Hills Armitage procedure, taking any carry-over effect into account, would be best for this study.

Response: Our study design was open-label, randomized, four-period crossover study. By choosing a crossover design where each patient served as his or her own control, we aimed to reduce the influence of inter-individual differences in regards to for instance insulin sensitivity.

Analysis of variance (ANOVA) allows us to analyze the differences between group means (insulin treatment groups). All the endpoints were log-transformed and analysed using ANOVA with treatment as a fixed factor and patient as a
random factor.
In this study, "carry-over" effects were avoided with a sufficiently long "wash-out" period (at least 7 days) between treatment visits. Further, we studied only one dose of different rapid acting insulin preparations. Thus, we believe carryover effects from one to the next the treatment are unlikely to occur.

Hills Armitage procedure is often used in the two-period cross-over design. It might not be an appropriate test for our study setting, and as we are unlikely to have a carry-over effect, we believe the ANOVA is appropriate.

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Editorial Requirements:
*Title Page: Please include on the title page, at minimum, the names, institutions, countries and email addresses of all authors, and the full postal address of the submitting author.
Response: We have added the requested information on the title page.

Reviewer's report:
Introduction
1. “Type 1 Diabetes”. Please use the term “Type 1 Diabetes Mellitus” and abbreviate as “T1DM” throughout the manuscript
Response: We found that ‘Type 1 Diabetes’ and ‘T1D’ were the preferred style in the BMC Endocrine Disorders.

2. “…as changes in bioactive IGF” Please provide an reference for the assumption that differences in IGFBP-1 may lead to changes in IGF (IGF1 or IGF2?)
Response: The previous reference No. 17 supports this assumption. This article is cited in this sentence. The reference number becomes No. 24.
We only state IGF bioactivity, avoiding I and -II. This is deliberately done, as both IGF-I and IGF-II are able to activate the IGF-I receptor in our in vitro test system as well as in vivo. Although we do believe the majority of the signal is caused by IGF-I, we cannot exclude that IGF-II is also of importance. Therefore, we have recently decided (after advice and intense debate) to use the phrase IGF bioactivity to acknowledge this fact.

3. The claim that alterations in the GH-IGF-1-IGFBP-1 axis may contribute to the development of long-term complications is still very speculative at the moment. The authors do provide 2 references. However, I would like to invite the authors to give some additional thoughts on this topic as this may improve the clinical significance of their findings.
Response: Thanks for this suggestion. We have by now modified the last half of the first paragraph of the introduction:

4. Please provide a testable hypothesis in the introduction or the patients and methods section.
Response: The hypothesis is specified in line 21, page 3.
Methods
5. The authors state that patients with impaired renal and hepatic function were excluded. How did the authors test this? Please provide the exact cut-off values for i.e. eGFR. How was a “recurrent major hypoglycaemia” defined?

Response: The renal (eGFR) and hepatic functions (pp, ALAT and alkaline phosphatase) were evaluated with patient’s medical record and routine lab tests in the past. The cut-off values were as stated by our Department of Biochemistry and these values are used at our hospital. This has been stressed in the text, line 19, page 4. We don't believe it is necessary to include these Danish cut-off values in the manuscript, but are willing to do so if requested.

Recurrent major hypoglycaemia was defined as a history of 2 or more major hypoglycaemic episodes (patient being unable to treat him/herself) within the last year prior to trial. This information was checked with patient’s journal and by asking patient in the screening visit. This has been added in the text, line 18, page 4.

6. What was the rationale for the 7-day interval between study visits?

Response: For the biphasic insulin aspart preparations, the duration of action of protaminated insulin aspart ranges from 14 to 24 hours. Thus, by choosing 7-day intervals between study visits we could eliminate any carry-over effect of the intermediary-acting insulin components. Secondly, by choosing only a week between study days, we reduced the possibility that the patient would change body composition, insulin sensitivity or physical behaviour (most Danes are more active in summer than winter time).

7. Did the patients receive any instructions for the period between the study phase/visits?

Response: Yes. Patients received detailed instructions regarding adverse events, insulin usage and study procedures etc. We highlighted and ensured that all patients were instructed not to inject the last dose of basal insulin at breakfast on the day proceeding the study day.

8. The authors state that “outcomes were controlled for baseline levels if necessary”. How was this performed?

Response: Baseline levels before each study day were analyzed with ANOVA and did not show any significant difference among four treatment groups. This indicates that the chosen time interval of 7 days between study days was appropriate. Baseline levels were used as co-variables in the ANOVA analysis for AUC0-3 and AUC0-9.

Results
9. Please provide standard deviation for the mean values.

Response: Before we prepared this manuscript, we reviewed the related literatures and found results were commonly presented as either mean + SD or mean & range. In this study, we used a 4-period, crossover design while patients served as their own control. Therefore, we believe using mean and range is a better way to show the general characteristics of recruited patients. In the table of
the result section, we also chose mean and range to present data since those results were drawn from ANOVA. Compared with mean + SD, this way is clearer for demonstrating the significant differences between any of two treatments. For instance, in the IGFBP-1 section, the reader can find that two ranges were apart if the differences between two treatments were statistically significant. However, we can provide mean and SD if you insist.

10. Did the patients use other medication than insulin?
Response: Yes. Some patients used the concomitant medications in this study. Those medications had been assessed and recorded by the investigator. None of the patients changed their other medications during the study. This has been added in line 20, page 4.

11. "Nineteen patients (15 men and four women)". Should be: Nineteen patients (15 men and 4 women). Please change this.
Response: Has been corrected

12. "…as defined a priori by the study protocol…'. Please remove "a priori". A study protocol is always a priori.
Response: This sentence is revised according to the comment.

13. Four patients had an extended profile day because glucose levels > 16 mM, were there any reasons for this? Were these patients included in the analysis?
Response: We referred to an early study for choosing this cut-off value. The effects of acute hyperglycaemia on cerebral function and cognitive performance were also taken into our consideration for patient safety. In ANOVA analysis, these patients were compared with available data. For instance, if a patient discontinued at 6 hours with insulin aspart treatment, his data were included for 0-3 h and 3-6 h analysis, not for 6-9 h analysis. This has been clarified in the revision, line 28, page 6.

14. Were there any correlations between IGF-1 bioactivity and total IGF-1 and IGFBP-1?
Response: Yes, changes in bioactive IGF and IGFBP-1 were inversely correlated as stated in results and as in Figure 3 and 4.

Discussion

15. The discussion is very well written. Nevertheless, as this study was not intended to investigate the safety of the different formulations of insulin the final conclusion of the authors that there is no reason to concern when using insulin aspart containing preparations is not justified. I would suggest to keep the conclusions mention in the discussion section in line with the, deliberate, conclusion in the abstract.
Response: We agree and have now deleted the safety part from the final conclusion section.

Reviewer's report:
The manuscript is well written, detailed and clear. It has a well defined aim and it
brings important information in the field of insulin treatment in type 1 diabetic patients and its effect upon the IGF-system. The methods are appropriate and well defined. The data is sound but it needs major corrections (see below). The conclusion and discussion are appropriate. The study limitations are clearly stated mainly in discussion section. The authors did not acknowledge the use of already printed data, but they described it in Methods and in the figure legend. The title and the abstract are linked to the results (major correction for abstract, see below).

Discretionary Revisions

1) Although it is well described that diabetes alters the IGF-system, did the authors consider to test samples from non-diabetic patients to compare the absolute values with samples from patients treated with the testing insulin preparations?
Response: This is a good suggestion, but we did not include healthy controls in this study. We will do this next time, and healthy controls are included in our ongoing studies.

Minor Essential Revisions

Figures:

2) Add statistical differences in all figures (for example, figure 2c).
Response: The statistical differences between mean curves were not calculated and compared with this study design. ANOVA with concentration as variance will be able to show these differences. However, in this study, we aimed to compare the concentration-time curve (AUC) with four insulin treatments in the same group of patients. Therefore, the AUCs represent variances in ANOVA rather than concentrations. The more detailed statistical analyses are shown in Tables 1. Figure 2c has been revised with p-value of AUCs as the significant differences were found in the comparisons.

Major Compulsory Revisions

Methods:

3) The patient features description are different in Results (paragraph 1) comparing with patient section in Methods (paragraph 1, patient section).
Response: the Method section describes the inclusion/exclusion criteria of this study. The Result section lists the characteristics of recruited patients.

Results:

4) The authors must confirm the values in the tables and the text. There are many results description that do not match with the indicated in the tables. For example, in abstract results section: “However, during the entire study, the area under the curve (AUC) of IGFBP-1 was higher following BIAsp50 (mean (range) 351, 312-396 µg*h/l) as compared to pure IAsp (262, 233-294 µg*h/l) and HI (256, 228-288 µg*h/l, p=0.001).” Those values correspond to AUC 0-3 h and not to AUC 0-9 h. Another example, in abstract results section: “Conversely, HI resulted in a lower AUC of IGFBP-1 than aspart containing preparations during
0-3, 3-6, 6-9 and 0-9 hours.” 6-9 h is not different among insulin preparations, as depicted by table 1.

Response: Thank you, we have now corrected inconsistencies. We have also shortened the abstract to remain within the word limit of 350.

5) The authors should consider citing more studies from others groups. Approximately, 1/3 of the cited articles seem to be from the authors group.

Response: We have tried to limit own citations and included data from other groups. However, some of our own citations are included solely as they describe the used assays. Further, as we originally developed the IGF bioassay, we have several relevant papers on this topic. Thus, it is hard to avoid citing own findings.