Author's response to reviews

Title: A prospective randomised cross-over study of the effect of insulin analogues and human insulin on the frequency of severe hypoglycaemia in patients with type 1 diabetes and recurrent hypoglycaemia (the HypoAna Trial): Study rationale and design

Authors:

Peter L Kristensen (pelk@hih.regionh.dk)
Ulrik Pedersen-Bjergaard (ulpebj@hih.regionh.dk)
Henning Beck-Nielsen (henning.beck-nielsen@ouh.regionsyddanmark.dk)
Kirsten Nørgaard (Kirsten.Noergaard@hvh.regionh.dk)
Hans Perrild (hans.perrild@dadlnet.dk)
Jens S Christiansen (JSC@KI.AU.DK)
Tonny Jensen (tonny.jensen@rh.regionh.dk)
Hans-Henrik Parving (hhparving@dadlnet.dk)
Birger Thorsteinsson (bith@hih.regionh.dk)
Lise Tarnow (ltar@steno.dk)

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Author's response to reviews: see over
Dear Editor

Thank you for your response to our article “A prospective randomised cross-over study of the effect of insulin analogues and human insulin on the frequency of severe hypoglycaemia in patients with type 1 diabetes and recurrent hypoglycaemia (the HypoAna Trial): Study rationale and design” (MS: 1196936506647499).

We find the comments from the reviewers relevant and have revised the manuscript accordingly. In the following we respond to their comments in detail. Red text indicates changes in the manuscript.

We hope that you find the revised version of the manuscript suitable for publication and are looking forward to hearing from you.

On behalf of all authors,

Yours sincerely,

Peter Lommer Kristensen

Comments to reviewer 1, Dereck Hunt

Page 2:
3rd last line in the “Background” paragraph, add “the” before the word “occurrence”. Accepted.

Page 4:
Line 5 under “Background”: add an “s” to the word “episode”. Accepted.
Line 6 under “Background”: change “much” to “, however, very”. Accepted.
Line 9 under “Background”: change “are” to “were” and change “in” to “with”. Accepted.
Line 14 under “Background”: change “less” to “decreased”. Accepted.

Page 5:
Last line: change “of” to “with”. Has not been changed.

Page 6:
Last line: change “in” to “with”. Accepted.

Page 8:
Line 8: change “at” to “on”. Accepted.

Page 12:
Line 7 under “Nocturnal hypoglycemia”: add “the” after the word “During”. Accepted.
Page 13:
First line under “HbA1c and plasma glucose”: change “totally” to “totaling”. Accepted.
Comments to reviewer 2, Paolo Rosetti (PR)

PR on the Introduction: “It should be clearly stated that the study focused only on some analogues (namely aspart and detemir). This is particularly relevant for insulin detemir. Indeed, there is some evidence in animals and also in healthy humans (2,3) that detemir may be associated with different responses to hypoglycaemia as compared to human insulin. Thus, results of the HypoANA trial cannot be extrapolated to other insulin analogues and this should be immediately made clear to the reader (minor essential revision).”

Comment from authors: We agree that the results of the study cannot be directly extrapolated to other insulin analogues and have made this more clear in the manuscript.

Revision in manuscript (page 6-7): The HypoAna Trial is designed to elucidate whether short-acting and long-acting insulin analogues (insulin aspart and insulin detemir) in comparison with human regular and NPH insulin are superior with respect to reducing the occurrence of severe hypoglycaemic episodes in patients with recurrent hypoglycaemia. There is an urgent need for this evidence in clinical practice and a need to elucidate if the higher costs of insulin analogues are justified in this respect. However, since the structures of different insulin analogues are not similar, the results of this study cannot be directly extrapolated to other insulin analogues (lispro, glulisine and glargine).

PR on the Introduction: “Objective. The same as Introduction: specify the type of insulin analogues used.”

Comment from authors: See the former comment and our answer.

Revision in manuscript: See the former comment and our answer.

PR on Methods, point 1.: “The Authors chose not to adopt a treat-to-target strategy for insulin titration. Instead, insulin doses were adjusted at discretion of the investigators. In my opinion this is the weakest point of the study. Indeed, the study is not blinded and not establishing common and strict titration rules for both the analogues and human insulins may well result in different metabolic control. This may be particularly relevant in the studied population where the fear of hypoglycaemia may influence either the investigators or the patients preventing effective insulin titration, especially in the human insulin group (human insulin is believed to cause more hypoglycaemia than insulin analogues). The consequence may be the achievement of suboptimal metabolic control and possibly lower-than-expected incidence of severe hypoglycaemia. However, as the study is already completed, results (metabolic control, incidence of hypoglycaemia) will tell us ex-post if
this methodological limitation affected the quality of the study. (As the study is completed, comments on this point are at the discretion of the Authors)."

**Comment from authors:** We acknowledge the dilemma raised by the reviewer. However, as he points out, the clinical part of the study is completed and no changes can be made to the protocol.

**Revision in manuscript:** None

**PR on Methods, point 2.**: “Were there any inclusion criteria regarding the pre-randomisation insulin treatment? Details regarding the pre-randomisation insulin treatment should be given (Minor essential revision)".

**Comment from authors:** There were no inclusion criteria regarding the pre-randomisation insulin treatment. All pre-randomisation insulin regimens were accepted and this has been added to the manuscript.

**Revision in manuscript (page 9):** The inclusion criteria are type 1 diabetes for more than five years, two or more episodes of severe hypoglycaemia in the previous year (defined as need for third party assistance to restore blood glucose level), age > 18 years, and a negative pregnancy test. There were no inclusion criteria regarding the pre-randomisation insulin treatment. Exclusion criteria are…

**PR on Methods, point 3:** “Severe hypoglycaemia (discretionary revision). For the sake of clarity and for the benefit of the non-expert reader, please when referring to the Whipple’s triad point out that biochemical definition of hypoglycaemia in patients with diabetes is different from the classical definition in healthy subjects.”

**Comment from authors:** We acknowledge that Whipple used the triad for the diagnosis of insulinoma during a supervised fasting test, using a crude assay for reducing substances as an indirect measure of blood glucose. Moreover, nowadays the blood glucose cut-off value for the definition of biochemical hypoglycemia, ranging from 2.8 to 4.0 mmol/l in different parts of the world of diabetes, is often somewhat lower for insulinoma, e.g. < 45-50 mg/dl (2.5-2.8 mmol/l). However, we find these remarks beyond the scope of our paper.

**Revision in manuscript:** None

**PR on Methods, point 4:** “CGM (minor essential revision). Details regarding the use of CGM and the intended analysis of its data should be provided. Was the CGM self-inserted by the patient and calibrated at home with capillary glucose? Or was it calibrated with venous plasma at the Hospital? This has an impact on the accuracy of blood glucose
estimations and should be specified. What metrics have the Authors planned to use? Time spent in hypo? What else? How have you planned to cope with the issue of false positive and false negative hypoglycaemia episodes as reported by the CGM?"

**Comment from authors:** The CGM was inserted at Steno Diabetes Center by a trained study nurse and calibrated with capillary glucose for the next three days. We are planning to report different CGM endpoints: Time spent at hypoglycaemia, number of episodes of mild hypoglycaemia and severe hypoglycaemia, different measures of variability, etc. Moreover, we are planning to do method studies of the accuracy of CGM in this selected patient population. Some sentences have been added to the manuscript.

**Revision in manuscript (page 12):** In the evening before bedtime, a study-specific blinded continuous glucose monitoring (CGM) device (Guardian® REAL-time, Medtronic Minimed, Northridge, USA) is mounted by a trained study nurse and the patient is instructed how to calibrate the device, using capillary glucose measurements. Thereafter, a venous line is inserted in an antecubital vein.

**Revision in manuscript (page 13):** Interstitial glucose concentrations are assessed for three days in patients who accept the four optional overnight stays at Steno Diabetes Center, i.e. 12 days of CGM in total. After the three days the glucose sensor and monitor are collected and data are downloaded to a computer for further analysis. These data remain blinded until the end of the study and are not used to control glycemic levels during the study period. Special blinded versions of the CGM device with a “black screen” were supplied by the manufacturer. The CGM data will provide a unique possibility to assess the accuracy of CGM and compare time spent at hypoglycaemia, number of unrecognized episodes of hypoglycemia in this selected patient population.

**PR on Methods, point 5.:** “Power calculation (minor essential revision). It is not clear how MIREDIF was established. The Authors should explain in what sense a 15% difference in the incidence of hypoglycaemia is considered meaningful. Are there data demonstrating (or at least suggesting) that a 15% reduction in the incidence of severe hypoglycaemia has an effect on hard endpoints (mortality, morbidity, cardiovascular events, etc.) or even on quality of life? Alternatively, does a 15% reduction in severe hypoglycaemia make insulin analogues cost-effective as compared to human insulin? If the Authors have planned to perform a cost-effectiveness analysis, details should be provided.”

**Comment from authors:** The MIREDIF at 15% is a mean of the 10-20% reduction which is stated as advantageous by the American Diabetes Association Workgroup on Hypoglycemia (American Diabetes Association Workgroup on Hypoglycemia: Defining and reporting hypoglycemia in diabetes. Diabetes Care 2005, 28:1245-1249). We are not planning to make cost-effectiveness analysis.

**Revision in manuscript (page 14):** Assuming an incidence of 2.8 severe hypoglycaemic events per patient-year, corresponding to 2.09 per 9 months treatment period, and setting minimal relevant difference (MIREDIF) to a reduction in the incidence of severe hypoglycaemia of 15% (based on the conclusion of the American Diabetes Association
Workgroup on Hypoglycemia "that any significant reduction in severe hypoglycemia (that requiring the assistance of another individual), even by as little as 10–20%, would be advantageous" [26]), this will correspond to a reduction in the relation between human and analogue insulin of: \( \lambda_{\text{insulin analogue}}/\lambda_{\text{human insulin}} = 0.85 \).

Comments to reviewer 3, James Shaw (JS)

**General remark by JS:** “It is noted that the study has already been completed and the Editors may wish to confirm that analysis has not been commenced prior to submission of this manuscript”.

**Comment from authors:** This has already been confirmed in an e-mail to Hayley Henderson from January 4. 2012.

**JS on Background, page 4 (1):** “…suggest ‘Insulin analogues have been developed ([Has not been changed, in conflict with Reviewer 1]) …Short acting insulin analogues…were designed ([Accepted])…long-acting insulin analogues were designed ([Accepted])…with minimal peak action ([Accepted])…with a presumed lower risk of hypoglycaemia ([Has not been changed, in conflict with Reviewer 1]) …for several reasons the impact of insulin analogues ([Accepted])…This renders the trials insufficiently…([Accepted])’

**Revision in manuscript:** As above indicated.


**Comment from authors:** The reference mentioned by JS is already cited in the original manuscript. However, the “author” *UK Hypoglycaemia Study Group* is added in the list of references.


Comment from authors: We agree that addition of the above mentioned reference is correct, although the article has been published after the termination of the present protocol. We find inclusion of the reference in the final article reporting the results of the HypoAna study mandatory.

Revision in manuscript: None

JS on Background, page 6: “Cite pilot study showing reduction in severe hypoglycaemia in type 1 diabetes complicated by impaired awareness of hypoglycaemia in an RCT including non-analogue MDI and short-acting analogue / glargine arms (A randomized pilot study in Type 1 diabetes complicated by severe hypoglycaemia, comparing rigorous hypoglycaemia avoidance with insulin analogue therapy, CSII or education alone. Thomas RM, Aldibbiat A, Griffin W, Cox MA, Leech NJ, Shaw JA. Diabet Med. 2007 24(7):778-83).

Comment from authors: We have added a few sentences in relation to the above mentioned reference.

Revision in manuscript: Although the risk of severe hypoglycaemia in women treated with insulin aspart was 28% lower, the difference did not reach statistical significance [24]. In a randomised 24-week pilot study of 15 patients with type 1 diabetes complicated by severe hypoglycaemia, comparing rigorous hypoglycaemia avoidance with insulin analogue therapy (lispro and glargine), continuous subcutaneous insulin therapy (CSII) or education alone (but not including any control group), hypoglycaemia awareness was restored and further severe hypoglycaemia was prevented with concomitant improvement in glycaemic control in the analogue and CSII groups [25].

JS on Background, page 7: “Suggest ‘insulin analogue and human insulin over the preceding 9 months…’ Accepted.”

JS on Methods, page 8: “Add further details on insulin regimens including number of injections per day and timing of basal insulins; whether prandial insulins were given before, during or after meals (number of injections per day), injection devices used and injection sites used”

Comment from authors: We have added a few sentences in relation to the above mentioned comment.

Revision in manuscript: The best obtainable glycaemic control for the individual patient is strived for in both treatment periods. The protocol did not specify the timing and number of insulin injections per day. In general, a four times daily basal-bolus regimen was selected, e.g. basal insulin before bedtime and prandial insulin before meals. Patients
were instructed in insulin injection technique at the beginning of each treatment arm. Endpoints are assessed...

**JS on Methods, page 9:** “Page 9: ‘hypothyroidism’ not ‘myxoedema’ (Accepted). Study ‘will be’ or ‘was’ conducted in accordance…” (Accepted). ‘Participants attend the outpatient clinic every three months’ (Accepted). After informed consent participants attend fasting…” (Accepted).

**JS on Methods, page 10:** “Suggest inclusion also of validated Clarke or Gold hypoglycaemia awareness questionnaires (clearly may now be too late). Why fasting C-peptide not stimulated or at least taken with concomitant plasma glucose with confirmation that this is not <4mmol/l.

**Comment from authors:** As the reviewer points out, inclusion of Clarke or Gold hypoglycemia awareness questionnaires is too late. However, The Pedersen-Bjergaard hypoglycemia awareness questionnaire is also validated and as effective as the Clarke or Gold questionnaires in identifying patients who prospectively will or will not experience episodes of severe hypoglycaemia (ref. 28.: Hoi-Hansen T, Pedersen-Bjergaard U, Thorsteinsnson B: Classification of hypoglycemia awareness in people with type 1 diabetes in clinical practice. Diabetes Complications 2010, 24:392-397). Fasting C-peptide and plasma glucose were measured simultaneously. Participants with C-peptide concentrations below 300 pmol/l or stimulated (venous blood glucose concentration >12 mmol/l) C-peptide concentrations below 600 pmol/l were considered C-peptide negative.

**Revision in manuscript:** These analyses are repeated after 1 year (before changing insulin regimen) and after 2 years (final visit). Participants with C-peptide concentrations below 300 pmol/l or stimulated (venous blood glucose concentration >12 mmol/l) C-peptide concentrations below 600 pmol/l were considered C-peptide negative. At every visit HbA1c and random...

**JS on Methods, page 10:** Was insulin given after breakfast?

**Comment from authors:** See “JS on Methods, page 8”.

**Revision in manuscript:** See “JS on Methods, page 8”.

**JS on Methods, page 11:** “Clarify whether 1987 or revised Adult Low Blood Sugar Survey version of Hypoglycaemia Fear Survey was used”.


Comment from authors: The 1987 version was used, since the revised version (HFS-II) was first validated in an article in 2011 (Gonder-Frederick LA, Schmidt KM, Vajda KA, Greear ML, Singh H, Shepard JA, Cox DJ. Psychometric properties of the hypoglycemia fear survey-ii for adults with type 1 diabetes. Diabetes Care (2011) 34:801-6.

Revision in manuscript: None.

JS on Methods, page 12: “Clarify whether truly blinded or real time CGM was employed”.

Comment from authors: Truly blinded CGM was employed. CGM-devices with “black” displays designed specifically for the Study were provided from Medtronic.

Revision in manuscript: (Guardian® REAL-time (with a “black” display), Medtronic Minimed, Northridge, USA).

JS on Methods, page 15: ‘repeated after 1 year (before changing insulin... (Accepted) is measured centrally at Steno... (Accepted) An ACTH stimulation test was undertaken... (Accepted) analyses are performed... (Accepted).