Author's response to reviews

**Title:** Short and long term outcome of neonatal hyperglycemia in very preterm infants: a retrospective follow-up study

**Authors:**

N. Margreth van der Lugt (margreth_vanderlugt@hotmail.com)  
Paul H.T. van Zwieten (p.vanzwieten@hagaziekenhuis.nl)  
Vivianne E.H.J. Smits-Wintjens (V.H.H.J.Smits-Wintjens@lumc.nl)  
Frans J. Walther (F.J.Walther@lumc.nl)

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**Author's response to reviews:** see over
Dear Editor:

Please find attached our revised manuscript. We appreciate the suggestions made by the three reviewers and have answered their questions below and changed the manuscript where appropriate (changes are marked in red).

We hope this manuscript is now acceptable for publication in BMC Pediatrics.

Sincerely,

Frans J. Walther

Responses to reviewers:

Reviewer: Anne van Kempen
Reviewer's report:
Major Compulsory Revisions
1. The main question is whether hyperglycemia by itself causes mortality, short term morbidity and long term neurodevelopmental problems or that hyperglycemia is an epiphenomenon of other neonatal diseases that increase neonatal morbidity and mortality and thereby influence long term neurodevelopment.

   The main difficulty of this study is that the chosen design makes it very complex to establish a reliable conclusion to the study question. A causal relationship between hyperglycemia and unfavorable outcome – as suggested throughout the report – is not proven by this study.

   Answer: Hyperglycemia is an expression of severe metabolic dysregulation in a preterm infant and a measure of the illness severity which will determine morbidity and mortality. We have tried to overcome this by comparing baseline characteristics and regression analysis. Insulin may indeed even have an adverse effect (see reviewer 3). We address this point in more detail in the discussion.

2. This study does not fulfill several criteria for the methodologic validity of prognostic studies:
   # Sample of subjects: Only hyperglycemic patients who were treated with insulin were included. Because the study was retrospective, the question is whether the decision to start insulin therapy was comparable in all patients. This could cause a selection bias. Second, it is unclear if the postnatal age was comparable at the time of hyperglycemia.

   Answer: The decision to start insulin therapy was protocol driven without a selection bias (hyperglycemia = at least 2 blood glucose values ≥ 10 mmol/l, insulin therapy is started after hyperglycemia persists for 12 hours at 0.05 U/kg/h. Insulin dosage was tapered after blood glucose values dropped to values < 10 mmol/l. Postnatal age at the time of hyperglycemia was 3.2 ± 3.7 days. This info was added to the methods section and table 3.
Because of the high mortality rate (27/66) and loss to follow up (1 excluded, 5 lost) the study group was quite small (n=33).

Answer: Indeed.

Most important: it is difficult to establish whether the control group is really similar to the insulin-treated group. Several important variables were addressed: the control group was matched according to gestational age and birth weight. Pre- and postnatal clinical conditions were reported like chorioamnionitis (although the used definition is questionable), sepsis, severe IRDS, severe IVH, PVL, NEC, BPD and pre- and postnatal steroids). All except BPD were comparable in the treatment and control group. However, length of stay and mortality were much higher in the treatment compared with the control group (47 vs. 26 days and 41% vs. 8%). These differences suggest that the infants in the treatment group had a more complicated neonatal course than the control group, which also could be responsible for their less favorable neurodevelopmental outcome. The authors state that this was the case in a subgroup of infants >1000 gram. More detailed information on especially the postnatal condition of the infants in both groups is needed, e.g. ventilation days, oxygen days, parenteral nutrition days, days of antibiotic therapy, CRIB score, etc.

Answer: The higher mortality and the longer length of stay and higher incidence of BPD in the exposed group are of course indicators of greater disease severity. We think that more data on ventilation etc do not add necessary information about this point.

3. Treatment with insulin is not unequivocally associated with better outcome. The first study in adults by van den Bergh showed very promising results. However, it proved to be difficult to repeat the positive results in later studies in adults, and some studies even showed negative effects. Early treatment with insulin in preterm infants (without hyperglycemia) even showed higher mortality at 28 days in the early-insulin group than in the control group (Beardsall NEJM 2008). The less favorable outcome in the insulin-treated group in this study could therefore also be due to the insulin therapy. This aspect must be addressed in the discussion.

Answer: We agree with the reviewer. Therapy might be worse than the disease… Reviewer 3 raises the same point which we now address in the discussion and in the conclusion.

Minor Essential Revisions

1. Units are missing in table 3.

Answer: Indeed, this omission was corrected.

Reviewer: Fabrizio BARBETTI
Reviewer's report:
It is not clear to this reviewer if the Authors can provide: 1) blood electrolytes and pH; 2) the number and severity of HYPOglycemic episodes, if any, possibly associated with insulin therapy.

Answer: We did not record blood electrolytes and pH systematically in our research database. The number of hypoglycemic episodes was added to table 3. Please note these were all one-time events which were corrected quickly.

I believe that without these two parameters it is really difficult to assess the impact of HYPERglycemia (or HYPOglycemia) on the outcomes studied, i.e. mortality and impact on neurological problems. In addition I wonder if it would be possible to separate infants with short-lived hyperglycemia (say 12-24 hours) from those with a longer (more severe ?), hyperglycemia and see if there is difference in mortality and/or neurological problems. It seems logical that hyperglycemia of 12 hours (the "minimum") can be not that relevant as compared to a hyperglycemia of 169 (i.e. 1 week) hours. The longer insulin therapy may also increase the chances of HYPOglycemic episodes.

Answer: We did not find a correlation between duration of hyperglycemia and outcome and added this to the results section. We added the incidence of hypoglycemia to table 3.

Other point. I think that it should be clearly stated in table 3 that "minimum" and "maximum" of duration are hours. If this is correct, then the text should be changed accordingly, because if maximum of insulin infusion was 754 (?) this mean that: 1) data analyzed are not of the first 5 days (page 8, first line) but longer, and 2) that insulin therapy lasted for some infants much longer than hyperglycemia. Can the Authors explain this ?

Answer: Indeed units were missing in table 3, this was corrected. Data were indeed analyzed beyond the first 5 days if the hyperglycemia lasted longer than 5 days. It is correct that insulin therapy lasted longer than hyperglycemia (blood glucose > 10 mmol/l) as the insulin dosage was tapered when blood glucose levels dropped below 10 mmol/l.

Reviewer: kathryn Beardsall
Reviewer's report:
Concerns to be addressed:
1. The rationale for the definition of hyperglycaemia used in this study is unclear as this is not one commonly used in the literature or known to have any clinical significance.

Answer: There is indeed no established definition of neonatal hyperglycemia but blood glucose levels above 7 mmol/l are usually considered to be high (Jane Hawdon in Robertson’s Textbook of Neonatology) and plasma glucose levels greater than 150 mg/dL (8.3 mmol/l) generally are considered to indicate hyperglycemia (Stanley and Pallott in Avery’s Diseases of the Newborn). In your recent article in ADC Fetal Neon Ed you write “In the preterm infant, the normally accepted levels for hypoglycaemia (<2.6 mmol/l) and hyperglycaemia (>10 mmol/l) occur frequently, particularly in infants born
<30 weeks or weighing <1500 g at birth (very low birth weight (VLBW)).” Our local protocols use blood glucose values ≥10 mmol/l as starting point for the definition of hyperglycemia in very preterm infants.

2. There is limited data on the frequency of blood glucose monitoring in either the ‘at risk’ or matched population to be sure that episodes of either hyperglycaemia or hypoglycaemia have not been missed. The clinical significance in a population being treated with insulin in terms of outcomes is important. This needs to be presented.

Answer: We added the following sentence to the methods section: During the first week of life blood glucose levels were measured at least 6 times a day, thereafter blood glucose levels were measured at least 3 times a day. In the acute phase of hyperglycemia, glucose levels were measured regularly with intervals of approximately 1-2 hours.

3. Why were data only collected for the first 5 days of the hyperglycaemic episode – was there any significant hyperglycaemia outside this period?

Answer: Data were collected throughout the hyperglycemic episode, we corrected this in the text on data collection “….were calculated for the first 5 days after the onset of the hyperglycemia episode or until the end of the hyperglycemia episode if the episode lasted longer than 5 days”.

4. Clinical criteria were for treating infants with insulin are not well presented statements such as ‘necessity of insulin treatment’ need clarifying – and this would be helpful for those wishing to know of the relevance to their own practice – similarly what were the criteria for stopping insulin?

Answer: Insulin treatment was deemed necessary if hyperglycemia persisted for more than 12 hours despite reduction of glucose intake to 5-6 mg/kg/min. This info was added to the methods section under the subheading study population.

5. It is not clear whether the controls were infants who did not have hyperglycaemia and the necessity for insulin treatment for >12 hours, but could have had unspecified periods of hyperglycaemia but not started on insulin or had insulin but for < 12 hours. – description of these infants would be helpful

Answer: The unexposed cohort was a matched selection of preterm infants admitted during the same timeframe, but without hyperglycemia (also without short-lived hyperglycemia of <12 hours) and insulin therapy. This info was added to the methods section.

6. Exclusion criteria – there is no explanation for why infants with parenchymal haemorrhage or infarction were excluded from the study – this needs to be discussed.
Answer: The reviewer is correct, these infants were placed back in the study. The text now states: The exclusion criteria for both exposed and unexposed infants were major congenital anomalies and chromosomal abnormalities.

7. The ‘behavioural outcome’ is not clearly described or referenced it is difficult to know what ‘inadequate’ behavioural outcome defines. This needs clarification.

Answer: Behavioural outcome was scored normal or inadequate based on the Child Behavior Checklist/2-3 (CBCL/2-3)[23] completed by the parents or orally by the clinic pediatrician. This was clarified in the text.

8. The data presented is not always consistent Table 1 n=33 however in Table 3 characteristics of glucose control are given for n=66.

Answer: We started out with 66 infants in the hyperglycemia group, after death and loss to follow-up we were left with 33 infants.

9. Infants with missing baseline characteristics were excluded from regression analyses – It would be helpful to know how many were excluded?

Answer: Sixty-one of the 859 very preterm infants had missing baseline characteristics. This was added to the results section. Fifteen of the selected 99 infants were excluded for regression analysis because of missing baseline characteristics. This info was added to table 2.

10. The multivariate regression analyses does not provide any information regarding the variables included in the model. In regression analyses it is usual to see if the outcome is related to other potentially confounding variables not to simply include only the variables that are statistically significantly different in the two study arms. For example IVH grade 3/4 may not statistically significantly different between the groups but it is likely to impact on outcomes and such relationships with outcomes should be explored -especially as there appear to be a higher incidence of IVH in those with hyperglycaemia. Similarly, regarding potential effect of hypoglycaemia.

Answer: “In the analyses for morbidity and mortality statistical correction was done for gestational age, gender, birth weight, exposure to prenatal and postnatal steroids, presence of severe RDS, sepsis, PROM, chorioamnionitis, PVL, BPD, NEC and IVH.” (last sentence of the results section). If we do the same for follow-up the difference in behavioural outcome is significant (p=0.039), but the difference for neurological outcome is not significant (p=0.083) because we lost 15/90 children with missing baseline characteristics. We now report our outcome data for follow-up as calculated by regression analysis, i.e. only differences in behavioural outcome.

**Additional points**
In the Abstract
i. The statement ‘Morbidity was more common in infants >1000g’ – is this really
true?

Answer: Yes, it is probably due to the general observation that these infants are more mature and have less “confounding” health problems in comparison with those <1000 g. The sentence was clarified by adding hyperglycemia vs without hyperglycemia.

ii. Statements that behavioural and development were more frequently abnormal among those with hyperglycaemia should be supported statistically

Answer: Indeed, p values were added.

iii. The conclusion gives the impression that the use of insulin treatment as opposed to the presence of hyperglycaemia is linked to mortality – this can not be concluded from this study.

Answer: We changed “in very preterm infants treated with insulin for hyperglycemia” into “in very preterm infants with hyperglycemia treated with insulin”.

Results
The details of the cohort would be better presented in a table 1 as an extra column

Answer: We think this information is not really relevant for this study.

Table 4 : This is reported to be multivariable regression analysis for mortality but it does not provide any information regarding what are the multiple variables included in the regression analyses? Were gestation and birth weight included as variables? The tables do not include all the necessary units

Answer: Please see the last sentence of the results section: “ In the analyses for morbidity and mortality statistical correction was done for gestational age, gender, birth weight, exposure to prenatal and postnatal steroids, presence of severe RDS, sepsis, PROM, chorioamnionitis, PVL, BPD, NEC and IVH.”

Discussion
Given the definition of hyperglycaemia including the use of insulin for >12 hours one can not assume that the association is due to poorly controlled hyperglycaemia in this design it may be the effect of insulin. This needs to be discussed. In addition the discussion would benefit by limiting to the data presented rather than hypothesis about animal data.

Answer: The animal data were taken out. We now also mention in the conclusion that outcome may be the effect of insulin instead of the presence of hyperglycemia.

Conclusions
Can not make any concluding comments about changes in brain structure, as this study does not report any data regarding brain structure and the conclusion would benefit from being more focused
Answer: We took out the concluding remarks about changes in brain structure and focused on the findings of the study.