Author's response to reviews

Title: Early Blood Glucose Profile and Neurodevelopmental Outcome at Two Years in Neonatal Hypoxic-Ischaemic Encephalopathy

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Author's response to reviews: see over
To the Editor,

Thank you most sincerely for giving us the opportunity to respond to the recommendations of the referees. We have addressed each of the comments as follow. We hope they are to your satisfaction and that of the reviewers.

Referee 1

Reviewer's report Title: Early Blood Glucose Profile and Neurodevelopmental Outcome at Two Years in Neonatal Hypoxic-Ischaemic Encephalopathy Version: 1
Date: 27 October 2010 Reviewer: Denis Azzopardi

Reviewer's report:

This is a report of a retrospective study comparing blood glucose values during the first 72 hours after birth with neurodevelopmental outcome at 24 months of age, in a cohort of 55 infants with hypoxic ischaemic encephalopathy. The authors found an association between a low blood glucose within 6 hours of birth and abnormal neurodevelopmental outcome. However although the authors stated that they performed multivariate logistic regression models it seems that only a univariate analysis was made of the relation between hypoglycaemia and outcome. The authors need to explain why a multivariate analysis including perinatal factors such as blood lactate, Apgar score, severity of HIE etc was not performed. A multivariate analysis is needed to explore for an independent effect of hypoglycaemia.

Response:

We are extremely grateful for the reviewer’s comments. We have reviewed our statistical analysis, and as predicted the relationship between early hypoglycaemia and outcome is no longer significant. Only grade of HIE remains a significant association.

The occurrence of early hypoglycaemia correlates significantly with severe HIE. This explains its association with abnormal outcome. We have adjusted our text to provide this information to the reader.

Comment: Minor comments are that the last two paragraphs of the introduction need correction and that the normal/abnormal outcome groups in figure 2 need to be more clearly separated.

Response:

Introduction and figure 2 have been amended.

Referee 2
The primary limitation, which the authors recognise but only partly discuss, is that this study cannot determine whether we should treat hypoglycaemia or not. The major reasons are of course partly just that this is an association, but more importantly that as shown by Salhab, that hypoglycaemia is more common with more severe encephalopathy. Given that in experimental studies of prolonged umbilical cord occlusion blood sugar falls it may well be that more severe insults cause lower sugars, not the other way around.1

In view of this it is critical that all therapeutic recommendations should be removed (repeated in several places with similar wording in the discussion), and appropriate warnings added to their conclusions, similar to those given by Salhab.

Response:

Thank you. The discussion and conclusion have been amended

Comment: It would greatly strengthen this paper to give the results of the full multivariate model including early hypoglycaemia as well as HIE severity, Apgar scores etc. Although the authors use multivariate logistic regression to show that the association of severity of HIE with primary outcome is not affected by glycaemic status, they do not tell us whether there is any independent effect of glycaemia. I suspect that there is not.

Response:

We have addressed this in the response to referee 1.

Comment: Minor amendments. The discussion of the limited experimental literature is confusing, because the authors don’t specify species, models and age. This is very important since there is evidence for example that intra-insult hyperglycemia is protective against hypoxia-ischemia in the infant rat 2,3, but not in the piglet 4. I am not aware of similar data in post-insult infusions, but the authors may wish to note that leblanc and colleagues found no effect of glucose infusion after HI in the term piglet, supporting their current study.

Response:

The discussion has been amended and we have added this information including 2 references (29, 30) to the manuscript

Comment: Table1. Please add mortality structured by severity of HIE.
Response:

Thank you. The table has been amended

Comment: How was repeated sampling accounted statistically for within each time period? Particularly in the severe group, babies may well have had multiple measurements.

Response:
The repeated samples were accounted by number of samples, not an average. HIE was graded by their clinical Sarnat score and it has been shown that 25, 18 and 9 infants had mild, moderate and severe HIE respectively. The mean (SD) number of blood glucose samples, taken in the first 72 hours, was 5.9 (2.9), 11.5 (5.7) and 13.1 (3.6) in infants with mild, moderate and severe HIE, respectively.

Comment: Trivia
Page 5 repeated “fetal brain ATP”
Ibid. 5000 what?

Response:

Thank you. The changes have been amended

Comment: Ibid. Since all children had HIE, I suspect that the criteria actually required evidence of HIE.

Response:

No definition of HIE is watertight, as the clinical markers which are often used are poor predictors of outcome, or severity of HIE. Acidosis, low Apgars, meconium staining have low positive predictive values: (Murray DM, Ryan CA, Boylan GB, Fitzgerald AP, Connolly S. Prediction of seizures in asphyxiated neonates: correlation with continuous video-electroencephalographic monitoring. Pediatrics. 2006;118(1):41–6).

Many of the published criteria for HIE are based on criteria which are not absolute requirements for hypoxic-ischaemic injury, such as multi- organ failure. This study
was a retrospective review of a prospective study of continuous EEG in infants with HIE. We therefore wished to recruit infants very soon after birth, and needed criteria which were obvious soon after birth: first pH, Apgar score, abnormal neurology. All infants had clinical and electrographic evidence of hypoxic-ischaemic encephalopathy, with abnormal neurology, a Sarnat score of mild, moderate or severe, and EEG evidence of encephalopathy consistent with the diagnosis. As such we have a very clearly defined cohort of infants with HIE. Most HIE studies are retrospective and based on unreliable markers. We are happy that our cohort represents infants with HIE.

We have reported the results of our prospective study in a number of recent publications. Please consider the following references


Comment: Page 7. Since 4 children were born at home, were these babies really able to have a blood sample within 30 min? If not, please present their time of first sample.

Response: In these infants, the samples were taken between 70-100 minutes. We have added this information to the result section.

Comment: Page 10. first para. ‘hypoglycemia reduced’ please change to specify was associated with etc.

Response: Sentence has been amended

Comment: Ibid. Please delete ‘nevertheless, hypoglycaemia should be avoided’ etc. This study does not address this issue.

Response:
Sentence has been amended

**Comment:** Page 10, bottom para, line 2. “part of EEG study” missing preposition.

**Response:** The sentence has been amended.

**Comment:** Page 11, top. What does definitive duration mean?

**Response:** We have removed this comment on duration.

**Comment:** Page 11. please delete “nevertheless, early hypoglycaemia “ etc.

**Response**
This has been amended

**Comment:** Figure 1. Please spread out data points so that they do not overlap. Many programmes will do this automatically.

**Response:**
The figure has been amended

**Comment:** How many datapoints can each child have at each time? Ie are their repeated samples here or have samples been averaged?

**Response:**
They are repeated samples, not average.

**Comment:** Figure 2. Please specify in the legend what the bars and whiskers represent.

**Response:**
The legend has been amended.

**Comment:**

**Response:**
Thank you for all these information. We have added references 2 and 5.

Referee 3  
Reviewer's report Title: Early Blood Glucose Profile and Neurodevelopmental Outcome at Two Years in Neonatal Hypoxic-Ischaemic Encephalopathy Version: 1 Date: 5 November 2010 Reviewer: Rosemary D Higgins Reviewer's report:

This manuscript describes the association of hypoglycemia with poor outcome following neonatal HIE.  
Compulsory revisions:  
**Comment:** 1. The “protocolized” blood glucose measurements appeared to only require a glucose measurement in the first 30 minutes of life. Can the author expand on subsequent glucose measurements and follow up protocol if the glucose was deemed low and IV dextrose was given beyond a follow up at 30 minutes to 1 hour?

**Response:**  
More than 1 bolus of dextrose infusion was required in four infants. All infants who required increased glucose infusions, experienced stable blood glucose within 2 hours of birth. Only 3 out of the 52 infants had 2 or more hypoglycaemic episodes recorded during the entire 72 hours of life. We have added this information to the results section.

**Comment:** Almost half (25) of the cohort had mild encephalopathy. These infants, based on the literature and the outcome described in the manuscript, would be expected to have fairly normal outcomes. Thus 30 infants are at risk for adverse outcome in the study making the numbers very small. Can the authors look at the glucose levels in the moderate and severe encephalopathy infants to determine if hypoglycemia adds to the risk over and above the level of encephalopathy for adverse outcome?

**Response:**  
We have addressed this in the response to referee 1.
Comment: In the section titled “HIE grade, glucose profile, and outcome,” it appears that HIE grade is more important in determining outcome. Was hypoglycemia observed more often in the moderate to severe encephalopathy group of infants?

Response:

Response: Mean (SD) of blood glucose levels did not differ between infants with mild, moderate or severe HIE. Mean (SD) of blood glucose level was Mean (SD) of blood glucose level was 4.7 (1.8), 4.9 (2.4) and 5.3 (2.5) mmol/L in infants with mild, moderate and severe HIE respectively, (p value 0.10). However early hypoglycaemia was more common in infants with severe encephalopathy and showed a significant correlation. We have added this information to the result section.

3. Were infants with hypoglycemia more likely to have seizures?

Response: The relationship between blood glucose levels and seizures was not the aim of our study and we have not examined this association.

4. Were there any deaths that occurred?

Response: Four infants died, we added this information to table 1. Death was included in the adverse outcome definition. Adverse outcome was defined as death, a Griffith’s Quotient (GQ) less than 87, or significant motor disability.

5. Were any children found to be blind, severely visually impaired, hearing impaired or deaf at follow up? If so, how were these variables used in the definition of adverse outcome at 24 months.

Response: Hearing and visual defects were assessed only in the context of Griffith scales. These scales contain five separate scales: locomotor, personal-social, hearing and speech, eye and hand coordination, and performance. The total developmental quotient (DQ)
and sub-quotients were calculated as described in the manual. No formal testing of hearing or visual acuity was performed at 2 years. We are currently examining this cohort at 5 years, with formal hearing and visual acuity assessment. However none of the infants were blind, severely visually impaired or had severe hearing impairment detected at routine screening at 2 years of age.

**Editorial requests:**

1) Please document that informed consent was obtained when the participants were enrolled in the study.

**Response:** We documented the same in the methods section.

2) Please structure your abstract according to our guidelines: http://www.biomedcentral.com/bmcpediatr/ifora/#abstract

**Response:** Abstract structure has been amended.

3) Authors contributions. We suggest the following kind of format (please use initials to refer to each author's contribution): AB carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript. JY carried out the immunoassays. MT participated in the sequence alignment. ES participated in the design of the study and performed the statistical analysis. FG conceived of the study, and participated in its design and coordination. All authors read and approved the final manuscript.

**Response:** Thank you very much. We have amended the authors’ contributions as you recommended.