PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Delayed impact of very low birth weight on glucose regulation: observational study
AUTHORS	Ryosuke Sato, Hiroshi Watanabe, Kenji Shirai, Shigeru Ohki, Rieko Genma, Hiroshi Morita, Eisuke Inoue, Masahiro Takeuchi, Masato Maekawa, Hirotoshi Nakamura

VERSION 1 - REVIEW

REVIEWER	Petteri Hovi Researcher Children's Hospital, University of Helsinki and the National Institute for Health and Welfare, Helsinki Finland I have no competing interests
REVIEW RETURNED	12/10/2011

THE STUDY	The research question 1. The research question or aim is to “clarify the delayed impact” of VLBW on glucose regulation. The design with no control subjects does not provide data on this. The only comparison to normal birth weight subjects is in the Discussion, it’s about HbA1% scores (Page 15), and it is too weak to support the structure of how this manuscript is currently written. The Methods 2. The numbers of those with impairments and those who died are needed both, instead for the sum of these. 3. More detailed reference need to be given regarding: A. the calculation of birth -weight percentiles. B. WHO criteria for IGT and IFG C. The estimated glomerular filtration rate needs to be clarified by telling on what measurements it bases on, how it is calculated, and where it is published. The abstract/summary/key messages/limitations 4. Article focus 2 gives the impression of absolutely no information on glucose regulation in adults born with VLBW. The term “uncertain” in focus 3 together with the text in focus 2 means “no information”. Instead of focus 2, I propose summarizing information on adult VLBW subjects 5. Asian population aspect comes a little late, in the Key messages. It might be involved in the Abstract\Methods? The references, 6. see above for three issues in Methods regarding inadequate references. 7. In the Introduction, 2nd paragraph “only a few studies” and then

	reference 8 alone are not in line. On the other hand, there are VLGA studies that might be referred to here as well due to large overlap between VLGA and VLBW groups. 8. When mentioning the reference 13, it should be noted that in that study the preterm subjects were compared to a term born group in which a large number of subjects were not likely to be representative of the underlying population. 9. The data provided by Ministry of Education,…including heights has to be included in the reference list. 10. The lowish rate (0.5) of VLBW births has to be specified (in which population) and supported by reference(s)
RESULTS & CONCLUSIONS	11. The study question should be formed again, see above.
REPORTING & ETHICS	12. In the Strobe 2007 checklist items 9 and 19 the authors state they have described their efforts of addressing potential bias, on page 8, and dealing with it, on page 16. However, I do not find the corresponding parts in the article and I think these issues are inadequately worked on in the whole article.
GENERAL COMMENTS	13. The authors, Ryosuke Sato et al., provide a nicely written text regarding their work on subjects born with VLBW with an OGTT-investigation in young adulthood. Unfortunately, this manuscript states the study question to be assessing any “impact” of VLBW on glucose regulation. Without a careful design involving control subjects, I find this study question very hard to tackle with. Instead, an article that would be more concentrated on the internal differences among the VLBW subjects could be more useful. For instance, the gender difference and the SGA-AGA comparison could be elaborated more, perhaps with a table that includes basic characteristics in the four subgroups by SGA and sex. However, some limitations in this study affect this approach as well; there are limited data on pregnancy, neonatal care, and subsequent growth. Some minor details that I think would improve the manuscript 14. The author’s definition of glucose intolerance needs to be defined in the abstract, before the Abstract\Results. In the text it should be again explained and accompanied with a reference, if possible. One detail about it is that impaired fasting glucose is included into it. Isn’t it so that you cannot measure “intolerance” unless you first administer something? 15. The results of the repeated measurements ANOVA are expressed in the Abstract and Results as “Men had… higher… glucose… and… lower… insulin..”. The text is summative. A more detailed approach is needed, or at least a referral to Figure 2, where the p-values are. 16. Figure 2 presents the sex difference in glucose and insulin measurements. From the figure it is apparent that the scores, of both glucose and especially insulin, are skewed. Therefore ANOVA and linear regression analysis could be run to logarithm transformed variables and the vertical axes could be in logarithm scale. Further, instead of presenting means, geometric means could be provided. The text on page 13 leaves it unclear whether some of the outcome variables were transformed before multiple linear regression. The clarified text suits best to the Methods. 17. A major limitation that could be included as a fourth bullet is that the study has no control subjects and it does not refer to any young adult OGTT data that would represent the situation in population. 18. “If prediction is possible” in the last sentence, first paragraph, does not, according to my judgement, fit into the style. 19. On page 12, “…BMI…a significant independent risk factor…” could be changed to “BMI is associated with”, since BMI and OGTT where measured at the same time.

	20. On page 16 the text can be interpreted as if the originally large number of subjects became as small as 111 entirely by the cautious exclusion process. Here, the participation rate would also be of interest. 21. In table 2, I cannot see how the odds ratios are to be interpreted. Males have 3.3 fold odds for “glucose intolerance” as compared to the women, that is clear. A 1-gram-increase in birth weight means a 1.00 (1.00 to 1.01) increase in the OR for glucose intolerance? I think this would be more informative if expressed as to kilograms. Where these Ors calculated in full models or by bivariate models, should be stated in the table footnote 22. In figure 1, instead of “detected” a more informative phrase would be “treated in the hospital”. 23. In figure 1, the authors could separate the “no response” from those “unwilling to participate” 24. Figure 2, see above and include more information on what the boxes, whiskers and dots stand for.

REVIEWER	D B Dunger Professor of Paediatrics Department of Paediatrics Box 116 Level 8 Addenbrooke's Hospital Hills Road Cambridge CB2 0QQ I do not have any conflicts of interest.
REVIEW RETURNED	19/10/2011

THE STUDY	Unclear if population studies is representative of VLBW population as it is only 18% of original VLBW population. ?demographic of babies not followed up. Not all the statistical methods are described in statistical methods section ie logistic and linear regression.
RESULTS & CONCLUSIONS	This is a very interesting study describing very high rates of impaired glucose tolerance (IGT in 111 young adults who were born with very low birth weight between 1980 and 1990. The occurrence of poor insulin secretion and IGT was particularly marked in males compared with females. Selection of subjects (111 out of 629 originally identified) is of concern as it may have introduced bias and some confirmation that this is a representative sample is essential. The lack of any control data is also of concern given the high rates of IGF/T2D reported in Japanese populations. Although the gender differences are intriguing it is impossible to avoid potential bias given that no data are presented around newborn, postnatal or childhood exposures. Thus although the data are potentially interesting, I cannot support publication without major revision.
GENERAL COMMENTS	Very interesting study describing high rates of impaired glucose tolerance (IGT) in 111 young adults who were born with very low birth weight between 1980 and 1990. The occurrence of poor insulin

	secretion and IGT was particularly marked in males when compared with females. A major limitation is the lack of appropriate controls although recent Japanese population data indicate that these rates of IGT are higher than expected. Selection of subjects is also a concern. Given that they have data on birth weight and gestation it is curious that they chose a broad WHO criteria for VLBW. They should use Japanese specific gestational and gender SDS data to further delineate small for gestational age infants. This did use in the analyses. Although the gender differences are intriguing it is impossible to avoid potential bias as no information between birth and adult life are available. The adult BMI data should be presented as an SDS to permit comparison degree of BMI differences between males and females. It would appear that the women are 'thin' but I am not aware of Japanese normative data. Correction for BMI SDS might reduce gender differences. Even if the observations are refined I suspect there will still be concerns about the lack of controls and the possibility of unknown bias. The author's test for counter regulation for response to reactive hypoglycaemia but this is not described. In addition, given the high incidence of NEC in this VLBW population, it would be important to know if those children who had reactive hypoglycaemia had required medical treatment or surgery for NEC which could impact on this outcome. Minor Point - rounding of p value's.

VERSION 1 – AUTHOR RESPONSE

Reply to Dr. Hovi

Dear Dr. Hovi:

Thank you for your kind and encouraging suggestions on strengthening our work. As indicated in the responses that follow, we have taken all comments and suggestions into account in the revised version of our paper.

To the specific comments:

1. Thank you for your helpful suggestion. As you mentioned, we have no control subjects born at term, which presents a limitation of the study. In the revised article, we have more focused on “the internal comparisons” within VLBW subjects than “impact of VLBW”. We have also changed the title of the manuscript to clarify the article focus. The modified title is as follows:

A cross-sectional study of glucose regulation in young adults with very low birth weight: impact of male gender on hyperglycemia.

2. We appreciate your constructive suggestion. As you commented, we have added the numbers of those with impairments and those who died (line 10 on p. 8). The sentence is as follows:

Out of the 628 subjects, 229 were excluded because of death (n=132) or severe neurodevelopmental

impairment (n=97).

3. According to your suggestion, we have added the following references.
A. The reference 10 (p. 21).
B. The reference 11 (p. 21).
C. The reference 17 (p. 22). The revised sentence is as follows (line 1 on p. 11)
The estimated glomerular filtration rate (eGFR) was calculated according to the following formula, as recommended by the Japanese Society of Nephrology: eGFR (ml/min/1.73m2) = 194 × Cre-1.094 × Age-0.287 (× 0.739 if the subject is a woman).

4. As per your kind advice, we have changed the Article focus 2 and 3 in the Article Summary (p. 5). The sentences are as follows:

ν Only a few studies have shown that VLBW (or preterm) is associated with glucose intolerance in Caucasian young adults, while glucose regulation in Asian young adults with VLBW is still uncertain.

ν The present study investigated glucose regulation in young adults with VLBW in an Asian population, and determined the factors associated with hyperglycemia.

5. We are grateful for your constructive suggestion. We have changed the sentences in the Article Summary (as mentioned above) to state that glucose regulation in Asian young adults with VLBW is still uncertain. We also stated the Asian population aspect in the Abstract (p. 3). The sentence we have revised in the Abstract is as follows:

(Abstract)
Objectives: To investigate glucose regulation in young adults with very low birth weight (VLBW; <1500g) in an Asian population.

6. As mentioned above (#3), we have added the references.

7. As per your comment, we have quoted another study of VLBW and VLGA (new reference 8; Diabetes Care 2011;34(5):1109-13) in addition to new reference 9 (former reference 8).

8. As you suggested, we have referred to the research (reference 13 in the original version) in the Discussion (line 1 on p.15). The changed sentences are as follows:

A recent epidemiological study has also shown that preterm birth is associated with an increased risk of diabetes in young adults. On the other hand, a study in the Netherlands showed that preterm birth was not associated with reduced insulin sensitivity in young adulthood. The findings of that study may be biased by the way of recruiting the control subjects born at term.

9. According to your suggestion, we have added the data provided by the Ministry of Education, Culture, Sports, Science, and Technology in Japan to the reference list (reference 28).

10. Thank you for constructive comment. The lowish rate (0.5) of VLBW birth is based on the statistics of the newborn data from 1980 to 1990 issued by the Ministry of Health, Labour, and Welfare in Japan. As per your comment, we have changed the sentence (line 5 on p. 17), and added this report to the reference list (reference 31).

The major strength of our study is a well-characterized cohort of subjects with VLBW, who are quite rare in the general population (approximately 0.5% in this generation).

11. As stated above, we formed the study question again (#1).

12. According to your suggestion, we have mentioned a potential selection bias that our study participants may not be representative of general healthy VLBW subjects (line 7 p.18). We have also changed the Strobe list items 9 and 19. The sentences in the manuscript are as follows:

The findings should be carefully interpreted taking into account the possibility that the participants might not be representative of general young adults with VLBW.

13. Thank you for your helpful advice. We have focused on the internal difference among the VLBW subjects and changed the title (as mentioned above: #1). As per your advice, we have changed Table 1, which includes basic characteristics, dividing the subjects into four subgroups by SGA and sex.

14. We appreciate your constructive suggestion. As you commented, we cannot measure "intolerance" unless we first administer glucose. According to your suggestion, we have removed the expression of glucose intolerance, and redefined this category as "a category of hyperglycemia". In the Abstract, we have also moved the definition of hyperglycemia to the section of "primary and secondary outcome measure" as per your suggestion.

15. As per your comment, we have changed the sentences of the results by the repeated measure ANOVA in the Abstract and the Results (line 2 p. 13). The sentences are as follows:

(Abstract/Results)
Male subjects had significantly higher levels of glucose and lower levels of insulin during OGTT than female subjects ($p<0.001$ for glucose and $p=0.005$ for insulin by repeated measure ANOVA).

(Results)
Male subjects had significantly higher levels of glucose during OGTT than female subjects ($p<0.001$ by repeated measure ANOVA). In terms of insulin levels, male subjects had lower levels of insulin during OGTT than female subjects ($p=0.005$ by repeated measure ANOVA).

16. As you mentioned, some variables in the study are skewed. We logarithmically transformed the following scores before the analyses; insulin levels (repeated measure ANOVA); and HOMA-IR, HOMA-β, Insulinogenic Index, and AUCglucose (linear regression model). As per your advice, in Methods, we have mentioned these variables logarithmically transformed (line 8 and 15 on p. 11). In terms of the values of insulin and glucose levels during OGTT in Figure 2, we had considered presenting the variables logarithmically transformed as your suggestion. However, for clinicians, it would be more useful to know the actual values rather than transformed values to make use of the data in practical care. Therefore, we chose to present actual values of glucose and insulin.
The sentences regarding the variables logarithmically transformed in Methods are as follows:

The data on insulin levels during OGTT were logarithmically transformed before the repeated measure ANOVA (line 8 on p. 11).

The data on HOMA-β, HOMA-IR, Insulinogenic Index, and GlucoseAUC were logarithmically transformed before analysis to meet the assumptions of normality (line 15 on p. 11).

17. As per your comment, we have mentioned that we have no control subjects born at term, and it is difficult to demonstrate the impact of VLBW on glucose regulation in Article Summary (p. 6), and the Strength and Limitation section (line 3 on p. 18). The sentence is as follows:

(Article Summary/Strength and limitations of the study)
The study design with no control subjects makes it impossible to address the delayed impact of VLBW itself on glucose regulation.

(Strength and weakness of the study)
In terms of subjects in the present study, our study has no control subjects, presenting a limitation of demonstrating the impact of VLBW itself on glucose regulation.

18. As per your suggestion, we have deleted the phrase, “If prediction is possible” (line 10 on p. 7). The revised sentence is as follows:

For this reason, to foresee the later risk of type 2 diabetes is very crucial for VLBW infants, which would lead to prevention of type 2 diabetes by early intervention in their lifestyle.

19. As per your advice, we have changed the phrase as follows (line 13 on p. 12).
BMI at study assessment was also associated with hyperglycemia

20. Thank you for the suggestion. As per your suggestion, we have added the information about the participation rate in the section of Strength and weakness of the study (line 5 on p. 18). We have also mentioned that our study participants may not be representative of general healthy VLBW subjects. The sentences are as follows:

Another concern of the study is selection bias. Of 301 VLBW subjects who were thought to receive the invitation letters, 111 subjects participated in the study (37%). The findings should be carefully interpreted taking into account the possibility that the participants might not be representative of general young adults with VLBW.

21. We calculated the odds ratio (OR) using the birth weight presented as to 1kg at first. The OR was 26.91; 95%CI: 0.634 to 1142.6; p-value=0.085. The OR was high and the range of 95%CI was so wide. Second, we estimated the OR using the value of birth weight presented as to 0.1kg in the logistic regression analysis. The calculated OR is 1.39 (95%CI: 0.96 to 2.02; p-value=0.085). We consider the latter value would be more informative, and noted this value in table 2. Thank you so much for the constructive suggestion.

22. As per your comment, we have changed the phrase to “treated in the hospital”.

23. We agree that it would be favourable to separate the “no response” from those “unwilling to participate”. However, no one clearly refused to participate in the study. It is impossible to detect individuals “unwilling to participate” out of all the non-responders. We have expressed all of them as “no response to the letters” in Figure 1.

24. As per your suggestion, we have added the information on what the boxes, whiskers, and dots stand for in Figure 2. The sentences are as follows:

The top and bottom of the box indicate lower and upper quartiles; the line inside the box represents the median; the whiskers indicate the most extreme data points within 1.5 times of interquartile range from the box; dots indicate outliers.

We greatly appreciate your kind and encouraging suggestions to our work. By virtue of your advice, our manuscript has been improved. We hope you will find the revised manuscript acceptable for publication in BMJ Open.

Yours sincerely,
Ryosuke Sato

Reply to Prof. D B Dunger

Dear Prof. D B Dunger:

Thank you for your kind and encouraging suggestions on strengthening our work. As indicated in the responses that follow, we have taken all comments and suggestions into account in the revised version of our paper.

To the specific comments:

Lack of control subjects
We appreciate your helpful suggestion. As you commented, the lack of control subjects presents a limitation of the study. As per your suggestion, we have mentioned that we have no control subjects born at term, and it is difficult to demonstrate the impact of VLBW itself on glucose regulation without control subjects in Article Summary (p. 6), and Strength and Limitation section (line 3 on p.18). The sentences are as follows:

(Article Summary/ Strengths and limitations of this study)
The study design with no control subjects makes it impossible to address the delayed impact of VLBW itself on glucose regulation.

(Strength and weakness of the study)
In terms of subjects in the present study, our study has no control subjects, presenting a limitation of demonstrating the impact of VLBW itself on glucose regulation.

Selection bias
As you mentioned, selection of subjects in the study may have introduced bias. This is our major limitation. We have mentioned that our study participants may not be representative of general healthy VLBW subjects (line 7 on p. 18). The sentence is as follows:

The findings should be carefully interpreted taking into account the possibility that the participants might not be representative of general young adults with VLBW.

Definition of VLBW and SGA
In the present study, we selected the WHO definition of VLBW (<1500g), which has been widely used in Japan as well as other countries in the world. In contrast, SGA was determined by a birth weight below the 10th percentile for gestational age according to standards defined by the Health Ministry in Japan (Reference 10). This definition of SGA is based on Japanese specific data.

Adult BMI SDS
Thank you for the suggestion. A report from the Japanese Ministry of Health, Welfare, and Labour in 2007 showed that the mean value of BMI in male general young adults (aged 20-29 years) was higher than that of females in the same generation (22.3 ± 3.4 kg/m2 vs. 20.3 ± 2.7 kg/m2). By contrast, in the present study, the mean value of BMI in male young adults with VLBW was lower than that of female VLBW subjects (20.5 ± 2.9 kg/m2 vs. 21.1 ± 4.2 kg/m2). As per your suggestion, we conducted a logistic regression analysis using adult BMI SDS instead of the BMI value itself. In the model adjusted for BMI SDS, family history of diabetes, gestational age, birth weight, and SGA/AGA,

male gender was still a statistically significant independent factor associated with hyperglycemia (OR: 5.76, 95%CI: 1.62- 20.5; p= 0.036). We consider that our vital finding, which suggests male gender is associated with hyperglycemia, is not skewed. Additionally, in other recent leading studies in this field focusing on adult subjects with low birth weight, adult BMI SDS was not necessarily used and BMI values were used for analysis (Hovi P et al. N Engl J Med 2007; 356(20): 2053-63/ Pilgaard K et al. Diabetologia 2010; 53: 2526-30).

Counter regulation/ NEC
We did not evaluate counter regulation for response to reactive hypoglycemia in the present study. We referred to counter regulation for the purpose of illustrating the definition of reactive hypoglycemia. In the study, reactive hypoglycemia during OGTT, which might be owing to delayed and excessive secretion of insulin (a possible early sign of glucose intolerance), was defined as the level of glucose less than 3.8 mmol/l that has been known to cause the response of counter-regulatory hormone release (Zammit NN et al. Diabetes Care 2005; 28 (12): 2948-61).
We did not refer to NEC in this article. We have no information regarding the past histories of NEC in this study.

Rounding of p-values
As per your suggestion, we unified the rounding of p-values in table 3.

We greatly appreciate your kind and encouraging suggestions to our work. By virtue of your advice, our manuscript has been improved. We hope you will find the revised manuscript acceptable for publication in BMJ Open.

Yours sincerely,
Ryosuke Sato

VERSION 2 – REVIEW

REVIEWER	David B Dunger Professor of Paediatrics Department of Paediatrics University of Cambridge Box 116 Level 8 Addenbrooke's Hospital Hills Road Cambridge CB2 0QQ, UK Tel 01223 336886/762943/769386 Fax 01223 336996 No I do not have any conflict of interest.
REVIEW RETURNED	08/12/2011

GENERAL COMMENTS	The authors have addressed the issues raised by the referees satisfactorily: They also acknowledge the limitations of their study due to lack of controls. A minor point: Clarification around percentage of original cohort studied - 37% responded to letters about follow up but not all of G28 were approached.

REVIEWER	Petteri Hovi Researcher Children's Hospital, University of Helsinki and the National Institute for Health and Welfare, Helsinki Finland I have no competing interests

REVIEW RETURNED	20/12/2011

GENERAL COMMENTS	I greatly appreciate the way in which the authors have worked in order to properly respond to my comments and requests. I really do not have anything crucial to add at this point, but I still would choose to express one of my minor concerns as follows: 3C. In the glomerular filtration formula, please include the units in which creatinine and age are to be when calculating. Keep all the formulas in the article uniform, to make them easy to read.

VERSION 2 – AUTHOR RESPONSE

Reply to Prof. D B Dunger

Dear Prof. D B Dunger:

Thank you for your kind and encouraging suggestions on strengthening our work. According to your favourable suggestion, we have revised the manuscript.

As per your suggestion, we have revised the sentence to clarify the percentage of the participant (p.18 line6). The sentence is as follows:

Of 301 VLBW subjects who were thought to receive the invitation letters, 111 subjects (37%) participated in the study.

In this study, the subjects born at VLBW were selected. The subjects were selected by the birth weight, not by the gestational age. This means that not all of them were born at G28.

We greatly appreciate your kind and encouraging suggestions to our work. We hope you will find the revised manuscript acceptable for publication in BMJ Open.

Yours sincerely,
Ryosuke Sato

Reply to Dr. Hovi

Dear Dr. Hovi:

Thank you for your kind and encouraging suggestions on strengthening our work. According to your favourable suggestion, we have revised the manuscript.

As per your suggestion, we have included the units of creatinine and age in the calculating formula, and have kept all the formulas in the article uniform to make them easy to read (p.11 line2, and p.25 line4).

We greatly appreciate your kind and encouraging suggestions to our work. We hope you will find the revised manuscript acceptable for publication in BMJ Open.

p.s. Thank you so much for your kind and favourable proposal to share our data. We are interested in

the Helsinki Study, and would like to collaborate with you for the progress in this field, if possible.

Yours sincerely,
Ryosuke Sato