PEER REVIEW HISTORY

BMJ Paediatrics Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Neonatal outcomes of very low birth weight infants born to mothers with hyperglycemia in pregnancy: a retrospective cohort study in Japan
AUTHORS	Hitaka, Daisuke; Morisaki, Naho; Miyazono, Yayoi; Piedvache, Aurelie; Nagafuji, Motomichi; Takeuchi, Syusuke; Kajikawa, Daigo; Kanai, Yu; Saito, Makoto; Takada, Hidetoshi

VERSION 1 – REVIEW

REVIEWER	Reviewer name: William W Hay, Jr. Institution and Country: University of Colorado School of Medicine USA Competing interests: None
REVIEW RETURNED	01-Apr-2019

GENERAL COMMENTS	The authors have done a large retrospective review of very low birth weight infants (about 28 weeks gestation, about 100 grams birth weight) whose mothers had documented hyperglycemia in pregnancy and found that the documented hyperglycemia was not associated with common morbidities of VLBW very preterm infants except for respiratory distress, and the respiratory distress only was noted to be increased before relaxed criteria for gestational diabetes were adopted, which the authors attributed to inclusion of more pregnant women with milder hyperglycemia. The large number of infants evaluated, the multicentered data, the focus on VLBW very preterm infants, and the assessment before and after GDM criteria were adopted are strengths of the study and report. A few other issues need further consideration. 1. A limitation of the study and report is that hyperglycemia in the mother was assessed primarily at one time point, around 24-28 weeks gestation. Risks of hyperglycemia are likely to be increased with persistent hyperglycemia, which was not assessed. This should be noted and commented upon. 2. Of the neonatal morbidities evaluated for associate with maternal hyperglycemia, only respiratory distress has been associated with maternal diabetes and hyperglycemia. It is not surprising therefore that no associations were noted between such morbidities and maternal hyperglycemia. 3. No data is provided about the prenatal condition of the mothers with respect to their degree of obesity (such as BMI), diabetes, hyperglycemia, insulin treatment, etc., all of which might have been associated with developmental abnormalities in the infants. 4. Other disorders of IDMs were not evaluated, and generally only occur in infants closer to terms, such as hyperbilirubinemia, polycythemia, hypocalcemia, and asymmetric cardiac septal hypertrophy.

	Since the focus of the study was on VLBW infants, it is not surprising that these morbidities were not found, but inclusion of infants later in gestation would have strengthened in the study and report. Perhaps the authors are presenting later gestation outcomes in another report. 5. Most of the macrosomia of IDMs develops in the 3rd trimester when maternal lipids as well as glucose produce increased fetal adiposity. It is not surprising, therefore, that prior to this physiological period of adipose tissue expansion there would not be an increase in fetal weight or BMI related to a one time measurement of maternal glycemia prior to the third trimester.

REVIEWER	Reviewer name: Nansi Boghossian Institution and Country: University of South Carolina, USA Competing interests: I have no competing interests to disclose.
REVIEW RETURNED	16-Apr-2019

GENERAL COMMENTS	In this paper, the authors examine neonatal outcomes of VLBW infants born to mothers with hyperglycemia during pregnancy in Japan. The authors report no associations with newborn outcomes except for RDS before the change in the IADPSG guidelines. Below are some comments: -Introduction: the authors point to the previous limitations in the literature in terms of the definition of diabetes being inconsistent in previous studies but the majority of their reported results have combined women diagnosed with hyperglycemia from before and after the change in the IADPSG criteria. -Intrauterine growth implies fetal growth which was not explored in this study. A better way of defining this is birth weight for gestational age. -Infants born after 34 weeks were excluded as these infants are more likely to be SGA to fit the VLBW cutoff. However, a large percentage of infants born at 33 and 34 weeks are also SGA. -Why were infants who died in the delivery room excluded? This might bias the findings especially if those who die in the delivery room are more likely to have mothers with hyperglycemia. -Was a regular Poisson regression used or a modified Poisson regression to estimate the RR? -Why did the authors adjust for SGA and Apgar-score at 5 minutes? Both of these factors are on the pathway from maternal hyperglycemia to neonatal outcomes and might result in overadjustment and bias the findings? -Why was a different p-value used for the interaction term (<0.1) versus <0.05 for the other reported findings? -Results section: the authors state they included infants 23 to 33 weeks but the table reports that infants <23 weeks and >34 weeks were excluded. Were infants of 34 weeks' gestation included or excluded? -How did the women excluded due to their characteristics and outcomes differ in terms of maternal hyperglycemia? -Page 15, discussion section, the reported reference (9-Boghossian et al.) is wrong when discussing the Persson et al paper. -The authors indicate that 'only the more severe cases of maternal hyperglycemia in pregnancy carry higher risk of RDS', why did the authors then continue to combine the two groups of women with hyperglycemia for all newborn outcomes i.e. combining those diagnosed with hyperglycemia before and after the change in the IADPSG criteria?

	-Discussion section: the authors state that their study had the power to detect the association between hyperglycemia in pregnancy and RDS due to the large sample size. However, the sample size of women with hyperglycemia was much smaller post change in the IADPSG criteria and might have resulted in the null findings. The authors need to examine the power and sample size to detect any differences post change in the criteria. -Discussion section: the authors state that the 'rate of RDS among infants of mothers with diabetes (62%) was comparable to other populations where only about 20% did not receive antenatal steroids'. However, this statement is not accurate as the selection criteria for these studies are very different. The NICHD NRN study for example included infants who are born with gestational ages between 22-28 weeks which is very different than what the authors included 23-34 weeks who have lower rates of RDS. -Women with hyperglycemia will be very different in terms of their newborn outcomes based on the severity of their hyperglycemia. Combining all these women will result in a very heterogeneous group and null findings.

REVIEWER	Reviewer name: M.M. van Weissenbruch Institution and Country: Department Pediatrics / IC Neonatology Location VUmc | Room number 8D61| De Boelelaan 1117, 1081 HV Amsterdam Competing interests: none
REVIEW RETURNED	25-Apr-2019

GENERAL COMMENTS	Neonatal outcomes of very low birth weight infants born to mothers with hyperglycemia in pregnancy: a multicenter study in Japan. Very interesting paper. However, I have some comments. Page 7 line 42/43: the data consisted of VLBW infants from over 95% of all level 3 NICUs in Japan (as of 2008). For me it is not clear from where the data derived from 2003-2008? Page 13 line 27/28: the proportions of mothers aged over 35 was significantly higher in mothers with hyperglycemia in pregnancy than in infants of mothers without it. I think there is a type error. Please delete "infants of" There is a difference in incidence in RDS in VLBW infants before the new guideline used in 2010. This is because of the change in criteria for GDM diagnose. I miss the following in the discussion: With the new diagnostic criteria are there any changes in counseling needed for parents with regard to morbidity especially of RDS in case of hyperglycemia in the mother in pregnancy? I believe no, because before the IADPSG guidelines for GDM diagnosis, VLBW infants born to mothers with severe hyperglycemia in pregnancy had an increased risk of RDS. With the limitations in mind, do you have any recommendation for the coming future regarding the antropometric data in infants. Figure 1. 104 deaths in the delivery room and congenital anomalies are these related to maternal hyperglycemia?

REVIEWER	Reviewer name: Franca Rusconi Institution and Country: Meyer Children's University Hospital Competing interests: None
REVIEW RETURNED	28-Apr-2019

GENERAL COMMENTS	In this study the authors examined the associations of maternal hyperglycemia in pregnancy and several neonatal outcomes in a cohort of very low birth weight infants in a multicenter study in Japan. Few papers are available on this topic in very preterm/very low birth weight infants. The present paper is undertaken in a large population of infants in a single country where the criteria for maternal hyperglycemia in pregnancy are likely to be uniform. The novelty of the paper lies in the analysis of the association between maternal hyperglycemia and outcomes before and after the adoption of the International Association of Diabetes in Pregnancy Study Group (IADPSG) guidelines in 2010 for diagnosis of gestational diabetes mellitus (GDM). Main points Introduction. Page 1. The “postition" of some phrases should be changed: the phrase “There have been only a few studies to examine the association between hyperglycemia in pregnancy and the mortality and morbidities of the premature infants” should be placed before “A recent study ….” In addition, it should be clear from the Introduction not only that there are few studies but also their inconsistent results, at least for the association with NEC. This should be added to the phrase on the few previous studies. The meaning of these small changes is to better contextualize the present study. Page 1, line 55: “Therefore it has been difficult to differentiate …”This phrase could be deleted; in fact in the context of studies undertaken in very preterm infants it is not difficult to differentiate whether the risk is due to prematurity or diabetes; the problem is to have a large number of pregnancies. Page 6, line 10. “…the effect… may differ by the severity of diabetes.” The authors should consider to add "and the impact of glycemic control mode on infant outcomes" citation number 9. Methods Page 10, line 52 ”Data on maternal diabetes were recorded except for the followings.. “ There is a long list of data that were not recorded but it is not clear which data were recorded on “maternal diabetes”. Results Title of table 3 should be changed in “Interaction between maternal hyperglycemia in pregnancy and periods (pre- and post-IADPSG) on several neonatal outcomes”. The text should also be changed reporting before the interaction results the number of mothers studied before and after IADPSG guidelines and the number with hyperglycemia in pregnancy. This information should be cancelled in table 3. Discussion

	Page 17, line 49: I am not sure that the authors could compare their results with those of Boghossian et al because no data on insulin use are available in the present study, and not even on diabetes diagnosis before pregnancy. This part of the discussion should be deleted Minor points A definition of VLBW infants should be added both in the abstract and in the Introduction. "What this study adds": the second phrase could be deleted, the third is enough for catching attention of the readers. Page 16, line 27 "…and this association persisted …" should be changed in "but this association was present only in…" Page 17, line 34 "It is universally known that term infants of maternal diabetes" should be changed in "that infants born at term from mothers with diabetes.." Page 19, line 6: "However statistical association" Please change in "Nevertheless an association …

REVIEWER	Reviewer name: Peter Flom Institution and Country: Peter Flom Consulting, USA Competing interests: None
REVIEW RETURNED	30-Apr-2019

GENERAL COMMENTS	I mostly confine my remarks to statistical aspects of this paper. The general approach is fine, but I have some issues to resolve before I can recommend publication. p 7 - bottom - So, which babies are left? That is, why are these babies VLBW if the are not very early, not SGA, no congenital anomalies etc.? p 9 Line 43 or so, Weren't SGA babies excluded? Line 55 - don't categorize mother's age. Categorzing continuous variables increases both type I and type II error. Leave age continuous and investigate nonlinearities with a spline.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

The authors have done a large retrospective review of very low birth weight infants (about 28 weeks gestation, about 100 grams birth weight) whose mothers had documented hyperglycemia in pregnancy and found that the documented hyperglycemia was not associated with common morbidities of VLBW very preterm infants except for respiratory distress, and the respiratory distress only was noted to be increased before relaxed criteria for gestational diabetes were adopted, which the authors attributed to inclusion of more pregnant women with milder hyperglycemia. The large number of infants evaluated, the multicentered data, the focus on VLBW very preterm infants, and the assessment before and after GDM criteria were adopted are strengths of the study and report. A few other issues need further consideration.

【A-1】

1. A limitation of the study and report is that hyperglycemia in the mother was assessed primarily at one time point, around 24-28 weeks gestation. Risks of hyperglycemia are likely to be increased with persistent hyperglycemia, which was not assessed. This should be noted and commented upon.

R: We appreciate and agree with the reviewer's comment. Indeed, we could not get the data on the type and onset of DM from the database we used. This has been commented in the Discussion section (Page18 Line33) as follows:

Furthermore, the mothers were assessed their glycemic status at one time point between 24 and 28 weeks of gestation, and thus we were not able to account for the increase in risks of hyperglycemia in proportion to its duration . Moreover, since most of the macrosomia of infants from diabetic mothers develops in the 3rd trimester, the assessment of anthropometric data from VLBW infants from diabetic mothers being checked once between 24 and 28 weeks of gestation may not be influenced thoroughly by glycemic status of their mothers.

【A-2】

2. Of the neonatal morbidities evaluated for associate with maternal hyperglycemia, only respiratory distress has been associated with maternal diabetes and hyperglycemia. It is not surprising therefore that no associations were noted between such morbidities and maternal hyperglycemia.

R: We agree with the reviewer's comment. In accordance with the reviewer's comment, we have added the following description in the Discussion section (Page 15, line 57): (their composite outcome did not differ between infants of mothers with or without diabetes.) With the exception of RDS, these null findings might be acceptable since associations between other severe morbidities and maternal hyperglycemia are not universally noted.

【A-3】

3. No data is provided about the prenatal condition of the mothers with respect to their degree of obesity (such as BMI), diabetes, hyperglycemia, insulin treatment, etc., all of which might have been associated with developmental abnormalities in the infants.

R: We agree with the reviewer's comment. Unfortunately in our study we lacked such maternal information, and we also excluded fetal abnormalities and were not able to assess risk of such developmental abnormalities. Following this comment, we have added the following description in the Discussion section (Page 18, line 27): (We acknowledge that there are several limitations to this study. First, we did not have data on the type and onset of DM, status of glycemic control, presence and details of treatment). Second, our study population was limited to preterm infants admitted to the NICU, thus excluding delivery room deaths, infants with congenital anomalies, and infants born at term. Thus, we were not able to evaluate disorders of IDMs such as hyperbilirubinemia, polycythemia, hypocalcemia, and asymmetric cardiac septal hypertrophy.

【A-4】

4. Other disorders of IDMs were not evaluated, and generally only occur in infants closer to terms, such as hyperbilirubinemia, polycythemia, hypocalcemia, and asymmetric cardiac septal hypertrophy. Since the focus of the study was on VLBW infants, it is not surprising that these morbidities were not found, but inclusion of infants later in gestation would have strengthened in the study and report. Perhaps the authors are presenting later gestation outcomes in another report.

R: We thank the reviewer's meaningful comment. Unfortunately our database only included infants with either birthweight under 1500 grams or gestational age under 32 weeks, and could not analyze on term births.

Following this comment, we have added the following description in the Discussion section (Page 18, line 27): (We acknowledge that there are several limitations to this study. First, we did not have data on the type and onset of DM, status of glycemic control, presence and details of treatment). Second, our study population was limited to preterm infants admitted to the NICU, thus excluding delivery room deaths, infants with congenital anomalies, and infants born at term. Thus, we were not able to evaluate disorders of IDMs such as hyperbilirubinemia, polycythemia, hypocalcemia, and asymmetric cardiac septal hypertrophy.

【A-5】

5. Most of the macrosomia of IDMs develops in the 3rd trimester when maternal lipids as well as glucose produce increased fetal adiposity. It is not surprising, therefore, that prior to this physiological period of adipose tissue expansion there would not be an increase in fetal weight or BMI related to a one time measurement of maternal glycemia prior to the third trimester.

R: Following re-analyses, we observed that birth weight in VLBW infants of maternal hyperglycemia in pregnancy was significantly higher than those in the control group. We have added in the discussion.

Most of the macrosomia of infants from diabetic mothers develops in the 3rd trimester, when maternal lipids as well as glucose produce increased fetal adiposity. Comparison of Boghossian's study and ours is difficult due to the paucity of detailed information on the types of DM and treatment in our study. One possible explanation to the difference in findings may be differences in prevalence of insulin therapy. While we do not have the exact proportion, considering the general prevalence of pregestational DM in Japan (<3%), it is likely that most women included in our study were not receiving insulin treatment prior to pregnancy. Our findings generate a hypothesis that mothers with hyperglycemia in pregnancy who do not require insulin before pregnancy deliver larger weight infants even if the delivery is very preterm, while those with more severe diabetes, who require the use of insulin before pregnancy, the infants tend to be smaller. Further research should be conducted on this topic.

Reviewer: 2

In this paper, the authors examine neonatal outcomes of VLBW infants born to mothers with hyperglycemia during pregnancy in Japan. The authors report no associations with newborn outcomes except for RDS before the change in the IADPSG guidelines. Below are some comments:

【B-1】

-Introduction: the authors point to the previous limitations in the literature in terms of the definition of diabetes being inconsistent in previous studies but the majority of their reported results have combined women diagnosed with hyperglycemia from before and after the change in the IADPSG criteria.

R: We appreciate and agree with the reviewer's comment. We have deleted and changed some phrase of the Introduction section (Page 6. Line18-28) as follows: However, the population combined those diagnosed with GDM from both before (2007-2010) and after (2010-2015) the publication of the International Association of Diabetes in Pregnancy Study Group (IADPSG) criteria for GDM in 2010.

【B-2】

-Intrauterine growth implies fetal growth which was not explored in this study. A better way of defining this is birth weight for gestational age.

R: We appreciate the reviewer's point, which we consider is also important. We have revised the introduction section to the following

Thus, the purpose of this study was to examine the association between maternal hyperglycemia in pregnancy and mortality, morbidities, birthweight for gestational age and extrauterine growth of VLBW infants and investigate whether this association differed before and after the adoption of the IADPSG criteria for GDM diagnosis.

【B-3】

-Infants born after 34 weeks were excluded as these infants are more likely to be SGA to fit the VLBW cutoff. However, a large percentage of infants born at 33 and 34 weeks are also SGA.

R: We appreciate and agree with the reviewer's point. In accordance with the reviewer's comment, we have excluded infants born at 33weeks and re-analyzed our data. As a result, 1533 infants have been excluded additionally from 31159 infants left for final analysis. This change (as well as other changes in the statistical analysis with use of spline model etc) have altered the result values, which is reflected in the revised manuscript. Here, we point out the significant changes.

-Average gestational age of infants of mothers with hyperglycemia in pregnancy was significantly higher than that of mothers without it (p=0.03). This has been stated as following:

(Page13, line33) Average gestational age of infants of mothers with hyperglycemia in pregnancy was significantly higher than that of mothers without it.

-The values of p-for-interaction of RDS and ROP from statistical test for interaction changed from 0.08 to 0.10, and 0.11 to 0.12, respectively. This has been stated as follows:

(Page14, line9) Statistical association between hyperglycemia in pregnancy and incidence of morbidities RDS and ROP of the infants significantly were almost differed close to significant between infants born in the pre- and post-IADPSG phase only for the incidence of RDS (p-for-interaction = .08 .10, .12, respectively) (Table 3). For both outcomes stratified analyses by study period were performed. As a result, for those born in the pre-IADPSG phase, infants of mothers with hyperglycemia in pregnancy had higher rates of RDS (RR 1.311.09, 95% CI 1.02-1.701.00 - 1.19) than infants of mothers without it. This difference was largely affected by infants born at 28 to 29 weeks of gestation (Figure in the Supplement). For those born in the post-IADPSG phase, there was no significant difference in the incidence of RDS between infants of mothers with and without hyperglycemia in pregnancy (RR 0.920.97, 95% CI 0.70-1.20 0.83 – 1.11). Regarding the incidence of ROP, there was no significant difference for those born in the pre- and post-IADPSG phase (RR 1.11, 95% CI 0.87 – 1.42, RR 0.85, 95% CI 0.64 – 1.11, respectively)

- Difference in weight z-score at discharge in infants of mothers with hyperglycemia in pregnancy became insignificant (p=.02 to .19) . Also, the change of weight z-score which was higher in infants of mothers with hyperglycemia in pregnancy compared with those of mothers without it became insignificant (P = .04 to .15). Phrases explained these had been deleted as follows:

(Page14, line33) Weight z-score at discharge in infants of mothers with hyperglycemia in pregnancy was higher than that of mothers without it (P = .02),

(Page14, line55) except in change of weight z-score which was higher in infants of mothers with hyperglycemia in pregnancy compared with those of mothers without it (P = .04).

【B-4】

-Why were infants who died in the delivery room excluded? This might bias the findings especially if those who die in the delivery room are more likely to have mothers with hyperglycemia.

R: We apologize for a lack of explanation on this part. Of the 104 infants who died at delivery room, only 1 infant born to mother with hyperglycemia in pregnancy, 99 infants born to non-diabetic mothers and 4 infants born to mothers without data on diabetes. These were analyzed before excluding 536 infants without data on maternal diabetes. Due to a significant uneven distribution of infants who died at delivery room, we had excluded them from the subject. To give a clear explanation, we have changed and added some phrase of the Results section (Page13. Line 6) as follows: Of these, 36920 infants were born at 23 to 33 weeks of gestation. Of these, 104 infants were excluded because of death at delivery room, 2043 infants were excluded because of congenital anomaly. In the remaining infants, 536 infants were excluded because of missing data on maternal diabetes and 3078 were excluded due to missing data on their characteristics and outcomes.

We have also changed the order of the corresponding part in Figure1.

【B-5】

-Was a regular Poisson regression used or a modified Poisson regression to estimate the RR?

R: We used modified Poisson regression (Poisson regression with a robust error variance).

【B-6】

-Why did the authors adjust for SGA and Apgar-score at 5 minutes? Both of these factors are on the pathway from maternal hyperglycemia to neonatal outcomes and might result in overadjustment and bias the findings?

R: We thank the reviewer's point. We included these variables as they have been used in previous studies as well, but in accordance with the reviewer's comment, we additionally conducted sensitivity analysis excluding SGA and Apgar-score at 5 minutes from the models, with no large change. The following are the items regarding RDS;

(Table 2) p value for incidence; 0.17 to 0.14

Adjusted relative risk and 95% CI; 1.04, 0.99-1.1 to 1.04, 0.99 -1.10 (remain the same)

(Table 3) p-for-interaction; 0.10 to 0.10 (remain the same)

【B-7】

-Why was a different p-value used for the interaction term (<0.1) versus <0.05 for the other reported findings?

R: We have followed the rule of relaxing the p-value to detect effect modification. "When assessing for effect modification, which is the impact of a third factor on the treatment-outcome relationship, it is increasingly acceptable to relax the α level to a higher value, typically 0.10 or 0.15, but in some instances as high as 0.20." (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5059270/)

【B-8】

-Results section: the authors state they included infants 23 to 33 weeks but the table reports that infants <23 weeks and >34 weeks were excluded. Were infants of 34 weeks' gestation included or excluded?

R: We apologize for the confusion. As responded above, we have excluded infants born at 33weeks, so infants being analyzed were born between 23 to 32 weeks. To carry a clearer message, we have replaced " > 34 week" with " ≥ 33 week" (Figure 1).

【B-9】

-How did the women excluded due to their characteristics and outcomes differ in terms of maternal hyperglycemia?

R: We are answering the question "How did the infants excluded due to their characteristics and outcomes differ in terms of maternal hyperglycemia?": Of 5761 infants excluded due to their characteristics and outcomes, 133 infants (2.3%) were born to mothers with hyperglycemia in pregnancy. The proportion of them were almost same compared to the 31159 infants we had analyzed. This does not change after excluding infants born at 33 weeks of gestational age.

【B-10】

-Page 15, discussion section, the reported reference (9-Boghossian et al.) is wrong when discussing the Persson et al paper.

R: We thank the reviewer's point. We have corrected the right reference (10-Persson et al.).

【B-11】

-The authors indicate that 'only the more severe cases of maternal hyperglycemia in pregnancy carry higher risk of RDS', why did the authors then continue to combine the two groups of women with hyperglycemia for all newborn outcomes i.e. combining those diagnosed with hyperglycemia before and after the change in the IADPSG criteria?

R: We thank our reviewer's point. We have revised our manuscript to explain that in all other outcomes other than RDS, even when stratified if there was a significant interaction, we failed to find a period-specific significant effect of maternal hyperglycemia in pregnancy.

【B-12】

-Discussion section: the authors state that their study had the power to detect the association between hyperglycemia in pregnancy and RDS due to the large sample size. However, the sample size of women with hyperglycemia was much smaller post change in the IADPSG criteria and might have resulted in the null findings. The authors need to examine the power and sample size to detect any differences post change in the criteria.

R: We agree that the smaller sample size may have led to the insignificant findings. However, we have based our comment of "the effect size differed post-era and pre-era" on fact that the interaction term was significant. We have refrained from using the term "we found a null effect" and used the wording "we were unable to identify any significant effect post-era."

【B-13】

-Discussion section: the authors state that the 'rate of RDS among infants of mothers with diabetes (62%) was comparable to other populations where only about 20% did not receive antenatal steroids'. However, this statement is not accurate as the selection criteria for these studies are very different. The NICHD NRN study for example included infants who are born with gestational ages between 22-28 weeks which is very different than what the authors included 23-34 weeks who have lower rates of RDS.

R: We apologize for confusing and agree with the reviewer's point. Indeed, mean gestational age of infants in our study with and without maternal hyperglycemia in pregnancy were 28.3 and 28.1 weeks respectively, which differed from those in NIHD NRN study (#9-Boghosssian et al, about 26weeks). On the other hand, mean gestational age of infants with and without maternal hyperglycemia in other 3 references we cited were not so different from ours (#6-Rehan et al; 29weeks and 29weeks, #7-Bental et al; 28.9weeks and 29weeks, #8-Grandi et al; 29.6weeks and 28.9weeks). We have corrected the citation numbers (#6 to #8) as the below (Page17, line 21):

….. where only about 20% did not receive antenatal steroids (6-8).

【B-14】

-Women with hyperglycemia will be very different in terms of their newborn outcomes based on the severity of their hyperglycemia. Combining all these women will result in a very heterogeneous group and null findings.

R: We agree with the reviewer's comment. Impossibility of differentiation the severity of maternal hyperglycemia is one of our study's limitation. In accordance with the reviewer's point, we have commented as follows in the Discussion section (Page 18 Line 33) as follows: First, we did not have data on the type and onset of DM, status of glycemic control, presence and details of treatment. Furthermore, the mothers were assessed their glycemic status at one time point between 24 and 28 weeks of gestation, and thus we were not able to account for the increase in risks of hyperglycemia in proportion to its duration. These paucity of information on maternal diabetes might lead to insufficient assessment of some risks such as NEC(8), in which Boghossian reported that preterm infants born to mothers with insulin use before pregnancy had higher risk of NEC than infants born to mothers with insulin use started during pregnancy and without insulin use.

Reviewer: 3

Neonatal outcomes of very low birth weight infants born to mothers with hyperglycemia in pregnancy: a multicenter study in Japan.

Very interesting paper.

However, I have some comments.

【C-1】

Page 7 line 42/43: the data consisted of VLBW infants from over 95% of all level 3 NICUs in Japan (as of 2008). For me it is not clear from where the data derived from 2003-2008?

R: We are sorry for confusing the reviewer. We have revised this part to carry a clearer message as follows; the data consisted of VLBW infants from 96% (72/75 as of 2008) of all level 3 NICUs and from 7 level 2 NICUs in Japan.

【C-2】

Page 13 line 27/28: the proportions of mothers aged over 35 was significantly higher in mothers with hyperglycemia in pregnancy than in infants of mothers without it. I think there is a type error. Please delete "infants of"

R: We thank the reviewer for pointing the mistake. This has been corrected.

【C-3】

There is a difference in incidence in RDS in VLBW infants before the new guideline used in 2010. This is because of the change in criteria for GDM diagnose.

R: We appreciate the reviewer's point. We cannot able to find any study which referred to the difference in incidence in RDS in VLBW infants before the new guideline used in 2010 as far as we searched.

【C-4】

I miss the following in the discussion: With the new diagnostic criteria are there any changes in counseling needed for parents with regard to morbidity especially of RDS in case of hyperglycemia in the mother in pregnancy?

I believe no, because before the IADPSG guidelines for GDM diagnosis, VLBW infants born to mothers with severe hyperglycemia in pregnancy had an increased risk of RDS.

R: We appreciate the reviewer's point, which we consider is also important. Indeed, we had described "Our finding that increased risk of RDS was observed only in infants of mothers with hyperglycemia in pregnancy diagnosed before the relaxation of the GDM diagnostic criteria in 2010, suggests that only the more severe cases of maternal hyperglycemia in pregnancy carry higher risk of RDS in infants. This finding is consistent with previous reports on mature infants, where RDS risk was found to be highest among those with the most severe cases of diabetes, namely, unstable type 1 diabetes (25)" in the Discussion section (Page 16, line33 to 49). As the reviewer noted, no change hade made on counseling needed for parents with regard to morbidity of RDS in case of hyperglycemia in pregnancy.

【C-5】

With the limitations in mind, do you have any recommendation for the coming future regarding the antropometric data in infants.

R: We thank reviewer's comment. It is well known that infants of diabetic mothers tend to be larger than infants of non-diabetic mothers. On the other hand, deterioration of diabetes mellitus in pregnant woman causes fetal growth retardation. Therefore, we would recommend analyzing the anthropometric data in infants born to diabetic mothers by severity of hyperglycemia. Furthermore, compared with term infants, preterm infants of diabetic mothers may not be fully affected by maternal diabetes due to the short time of intrauterine life. We cannot find how to address this issue at this time. Incidentally, in accordance with other reviewer's point, this has been commented in the Discussion section (Page18 Line33) as follows:

Our findings generate a hypothesis that mothers with hyperglycemia in pregnancy who do not require insulin before pregnancy deliver larger weight infants even if the delivery is very preterm, while those with more severe diabetes, who require the use of insulin before pregnancy, the infants tend to be smaller. Further research should be conducted on this topic.

【C-6】

Figure 1. 104 deaths in the delivery room and congenital anomalies are these related to maternal hyperglycemia?

R: We thank reviewer's question. We cannot get any information whether maternal hyperglycemia was related to delivery room deaths and congenital anomalies because of the design for the database we used. We should mention this as a study limitation. Therefore, we have added the following description in the Discussion section (Page 18, line 42):

Second, our study population was limited to preterm infants admitted to the NICU, thus excluding delivery room deaths, infants with congenital anomalies, and infants born at term. Thus, we were not able to evaluate disorders of IDMs such as hyperbilirubinemia, polycythemia, hypocalcemia, and asymmetric cardiac septal hypertrophy.

Reviewer: 4

In this study the authors examined the associations of maternal hyperglycemia in pregnancy and several neonatal outcomes in a cohort of very low birth weight infants in a multicenter study in Japan.

Few papers are available on this topic in very preterm/very low birth weight infants. The present paper is undertaken in a large population of infants in a single country where the criteria for maternal hyperglycemia in pregnancy are likely to be uniform. The novelty of the paper lies in the analysis of the association between maternal hyperglycemia and outcomes before and after the adoption of the International Association of Diabetes in Pregnancy Study Group (IADPSG) guidelines in 2010 for diagnosis of gestational diabetes mellitus (GDM).

Main points

Introduction.

【D-1】

Page 1. The "position" of some phrases should be changed: the phrase "There have been only a few studies to examine the association between hyperglycemia in pregnancy and the mortality and morbidities of the premature infants" should be placed before "A recent study …."

In addition, it should be clear from the Introduction not only that there are few studies but also their inconsistent results, at least for the association with NEC. This should be added to the phrase on the few previous studies. The meaning of these small changes is to better contextualize the present study.

R: In accordance with the reviewer's comment, we have changed the position of phrase Page 5, Line 42 "There have been only a few studies to examine the association between hyperglycemia in pregnancy and the mortality and morbidities of the premature infants" to before Page 6, Line9 "A recent study conducted in 7 countries (including Japan)….". And we have placed the additional phrase Page6 "Several studies had reported that VLBW infants born to mothers with hyperglycemia in pregnancy were at higher risk of necrotizing enterocolitis (NEC), though others had not shown"

【D-2】

Page 1, line 55: “Therefore it has been difficult to differentiate …”This phrase could be deleted; in fact in the context of studies undertaken in very preterm infants it is not difficult to differentiate whether the risk is due to prematurity or diabetes; the problem is to have a large number of pregnancies.

R: We appreciate and agree with the reviewer’s comment and the phrase has been deleted. To arrange the context, we have changed Page 6, Line 6: “To further complicate the matter” to “Furthermore, “

【D-3】

Page 6, line 10. “…the effect… may differ by the severity of diabetes.” The authors should consider to add "and the impact of glycemic control mode on infant outcomes" citation number 9.

R: In accordance with the reviewer’s comment, we have added the sentence and cited the reference.

Methods

【D-4】

Page 10, line 52 ”Data on maternal diabetes were recorded except for the followings.. “ There is a long list of data that were not recorded but it is not clear which data were recorded on “maternal diabetes”.

R: We are sorry for confusing the reviewer. To clarify the meanings, this part has been changed as follows: Data on the presence of maternal glucose intolerance during pregnancy was recorded except for all of the following items.”

Results

【D-5】

Title of table 3 should be changed in “Interaction between maternal hyperglycemia in pregnancy and periods (pre- and post-IADPSG) on several neonatal outcomes”.

The text should also be changed reporting before the interaction results the number of mothers studied before and after IADPSG guidelines and the number with hyperglycemia in pregnancy. This information should be cancelled in table 3.

R: We appreciate and agree with the reviewer’s comment. This has been corrected in accordance with the reviewer’s comment. The first 2 phrase in legend has been deleted.

Discussion

【D-6】

Page 17, line 49: I am not sure that the authors could compare their results with those of Boghossian et al because no data on insulin use are available in the present study, and not even on diabetes diagnosis before pregnancy. This part of the discussion should be deleted

R: We agree with the reviewer in that we could not compare our results with those of Boghossian et al. On the other hand, we think it important to discuss about a relationship between infants’ anthropometric data and hyperglycemia in pregnancy. To carry a clearer message, we have revised this part of the Discussion section (Page 17, line 33 to Page 18, line 24, whole 6th paragraph of the discussion section) as follows:

It is universally known that term infants of maternal diabetes infants born at term from mothers with diabetes tend to have larger birth weights (unless the disease is severe); however, anthropometric data of preterm infants of maternal diabetes is lacking. In our study, birth weight in VLBW infants of maternal hyperglycemia in pregnancy was significantly higher than those in the control group. Our study findings differed from those from Boghossian who reported that extremely preterm infants of mothers using insulin before pregnancy were smaller at birth (height and head circumference, but not weight) than mothers who do not use insulin before pregnancy, which the author attributed to prolonged hyperglycemia causing deterioration of vascular condition (8). Most of the macrosomia of infants from diabetic mothers develops in the 3rd trimester, when maternal lipids as well as glucose produce increased fetal adiposity. Comparison of Boghossian's study and ours is difficult due to the paucity of detailed information on the types of DM and treatment in our study. One possible explanation to the difference in findings may be differences in prevalence of insulin therapy. While we do not have the exact proportion, considering the general prevalence of pregestational DM in Japan (<3%), it is likely that most women included in our study were not receiving insulin treatment prior to pregnancy. Our findings generate a hypothesis that mothers with hyperglycemia in pregnancy who do not require insulin before pregnancy deliver larger weight infants even if the delivery is very preterm, while those with more severe diabetes, who require the use of insulin before pregnancy, the infants tend to be smaller. Further research should be conducted on this topic.

If the reviewer still recommends a deletion of this part, we are ready to follow.

Minor points

【D-7】

A definition of VLBW infants should be added both in the abstract and in the Introduction.

R: In accordance with the reviewer's comment, we have added "<1500 grams" at Page2, line12 and Page5.

【D-8】

"What this study adds": the second phrase could be deleted, the third is enough for catching attention of the readers.

R: We appreciate and agree with the reviewer's comment. Second phrase has been deleted.

【D-9】

Page 16, line 27 "…and this association persisted …" should be changed in "but this association was present only in…"

R: We appreciate and agree with the reviewer's comment. This has been corrected in accordance with the reviewer's comment.

【D-10】

Page 17, line 34 "It is universally known that term infants of maternal diabetes" should be changed in "that infants born at term from mothers with diabetes.."

R: We appreciate and agree with the reviewer's comment. This has been corrected in accordance with the reviewer's comment.

【D-11】

Page 19, line 6: "However statistical association" Please change in "Nevertheless an association …

R: We appreciate and agree with the reviewer's comment. This has been corrected in accordance with the reviewer's comment.

Reviewer: 5

I mostly confine my remarks to statistical aspects of this paper. The general approach is fine, but I have some issues to resolve before I can recommend publication.

【E-1】

p 7 - bottom - So, which babies are left? That is, why are these babies VLBW if they are not very early, not SGA, no congenital anomalies etc.?

R: We apologize for the confusion. We have revised this part to carry a clearer message as follows; (Page 7, line 54) We also excluded VLBW infants born between 23 to 32 weeks with congenital anomalies (serious congenital heart disease or major genetic disorder), those who died at the delivery room, and those with missing data on maternal diabetes, or missing data on their characteristics and outcomes.

Appendix: In response to another reviewer, we have excluded infants born at 33 weeks of gestational age due to the dominance of SGA.

【E-2】

p 9 Line 43 or so, Weren't SGA babies excluded?

R: We apologize for the confusion. We had excluded VLBW(<1500g) infants born after 33 weeks since almost of them were relatively small for their gestational weeks. On the other hand, SGA infants (birth weight being less than the 10th percentile for the gestational age) born between 23 to 32 weeks were not excluded and we had regarded it as a confounder when conducting statistical analysis.

Appendix: In response to another reviewer, we have excluded infants born at 33 weeks of gestational age due to the dominance of SGA.

【E-3】

Line 55 - don't categorize mother's age. Categorzing continuous variables increases both type I and type II error. Leave age continuous and investigate nonlinearities with a spline.

R: In accordance with the reviewer's comment, we have left mother's age continuous and performed a spline analysis. We have deleted "Regarding maternal characteristics, maternal age was categorized into three categories: under 20, 20 to 34, and over 35." (Page 9, line54) and added "Maternal ages were left continuous and investigated non-linearities using cubic spline regression model." (Page 11, line57)

This method has changed our results shown in Table2, Table 3, and Table4, which were also affected by other alteration in the way of statistical analysis in correspondences with other reviewers (excluding infants born at 33 weeks of gestational age, etc). Here, we point to significant changes as below.

-The values of p-for-interaction of RDS and ROP from statistical test for interaction have been changed from 0.08 to 0.1 ,0.11 to 0.12. This has been stated as follows:

(Page14, line9) Statistical association between hyperglycemia in pregnancy and incidence of RDS and ROP of the infants were almost close to significant between infants born in the pre- and post-IADPSG phase (p-for-interaction = .10, .12, respectively) (Table 3). For both outcomes stratified analyses by study period were performed. As a result, for those born in the pre-IADPSG phase, infants of mothers with hyperglycemia in pregnancy had higher rates of RDS (RR 1.09, 95% CI 1.00 - 1.19) than infants of mothers without it. This difference was largely affected by infants born at 28 to 29 weeks of gestation (Figure in the Supplement). For those born in the post-IADPSG phase, there was no significant difference in the incidence of RDS between infants of mothers with and without hyperglycemia in pregnancy (RR 0.97, 95% CI 0.83 – 1.11). Regarding the incidence of ROP, there was no significant difference for those born in the pre- and post-IADPSG phase (RR 1.11, 95% CI 0.87 – 1.42, RR 0.85, 95% CI 0.64 – 1.11, respectively)

-Significant difference in weight z-score at discharge in infants of mothers with hyperglycemia in pregnancy have disappeared (p=.02 to .19) . Also, significant difference of the change of weight z-score which was higher in infants of mothers with hyperglycemia in pregnancy compared with those of mothers without it had disappeared (P = .04 to .15). Phrases explained these had been deleted as follows:

(Page14, line33) Weight z-score at discharge in infants of mothers with hyperglycemia in pregnancy was higher than that of mothers without it (P = .02),

(Page14, line55) except in change of weight z-score which was higher in infants of mothers with hyperglycemia in pregnancy compared with those of mothers without it (P = .04).