PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Relationship between maternal hypoglycaemia and small-for-gestational-age infants according to maternal weight status: a retrospective cohort study in two hospitals
AUTHORS	Shinohara, Satoshi; Uchida, Yuzo; Hirai, Mitsuo; Hirata, Shuji; Suzuki, Kohta

VERSION 1 - REVIEW

REVIEWER	Sachiko Baba Osaka University, Japan
REVIEW RETURNED	22-Aug-2016

GENERAL COMMENTS	This study examined the association of hypoglycemia and SGA infant stratified by maternal pre-pregnancy BMI in Japan. The study was well documented that low GCT result in the 2nd trimester are associated with SGA infant among underweight Japanese women in two hospitals in Japan. Major comments 1. The outcome is defined as SGA “infant” in this study, but generally SGA could be identified during pregnancy using ultrasound, at least in high-income countries. It is well demonstrated in this study that hypoglycemia in the 2nd trimester is associated with SGA at delivery, but is this finding useful to identify the SGA risk during pregnancy in addition to conventional method using ultrasound ? 2. Also, if GCT has low reliability that author stated on page 14 line 43, the importance of this finding in this study may be low. Please explain why the estimation of SGA infant using GCT in the 2nd trimester is important. 3. FGR and SGA is not identical. Please clarify the usage of these words in the manuscript. Minor comment Title 4. a study with two hospital could be called as “multi-centre study”? Abstract 5. The first sentence is not similar to what is described in the title. Please check. Background Method

	6. Is it reasonable to analyze to combine preterm SGA and term SGA? 7. Maternal weight gain was used as a confounding factor, but it is dependent on gestational weeks. Moreover, desired maternal weight gain is also depend on pre-pregnancy weight. If you insist on using maternal weight gain as a variable, you should use weight gain per week like reference 16 instead of gross maternal weight gain. However, it is recommended to consider again whether maternal weight gain is appropriate for a confounder. 8. On page 6 line 41-48, authors described confounding variables by citing reference 5, 15, and 16. However, references 5 and 15 are studies among Japanese women. How about other studies in the U.S. and European countries? Reference 6 is a study in the U.S. among women less than 25 years old but the variables used are not identical to this study. 9. Maternal height could be added as a confounder even though pre-pregnancy maternal BMI is put into the model. Result 10. In Table 2, the outcome variable (low GCT) should be put in the first place. 11. Discussion 12. The generalized word “hypoglycemia” could be used not only in the title but also in discussion. 13. Please explain whether smoking or other unused variables described in the limitation section is associated with the result of this study and insulin sensitivity/ resistance. 14. In the background, authors hypothesize the insulin resistance is developed late at pregnancy determined by the pre-pregnancy BMI, but in the discussion, authors discuss high insulin sensitivity at early pregnancy.

REVIEWER	David B. Dunger University of Cambridge, U.K.
REVIEW RETURNED	26-Aug-2016

GENERAL COMMENTS	In this study the authors observed that the association between maternal plasma glucose concentrations, one hour after an oral 50 g glucose load, during late pregnancy and the incidence of SGA offspring, appears to be entirely confined to underweight women. The manuscript adds very little to the authors’ original observation (citation [8] in the manuscript). Although the manuscript analyses results from a very slightly larger cohort the results are very similar and the ‘new’ data relates to the evaluation of maternal BMI. Although much is made about the size of the study, because of the sub-analyses the study is probably underpowered. For examples, in Table 2 with maternal BMI <18.5 there were only 7 women with thyroid disease and 8 women with PIH, for women with BMI > 25 there were only 2 teenage pregnancies and 4 women with thyroid disease. These numbers are too small to draw meaningful conclusions. The limitations of the study should be recognised. There are frequent references to ‘insulin resistance’ and ‘foetal growth restriction’ but no data are provided for either variable. It is not clear why pregnancy BMI was not used as a continuous variable in the analysis of the relationship between maternal glucose and birth

	weight – could maternal age be a further confounder? Finally, the generalisability of the data to non-Japanese populations should be discussed.

VERSION 1 – AUTHOR RESPONSE

RESPONSE TO REVIEWER 1

Comment 1
The outcome is defined as SGA “infant” in this study, but generally SGA could be identified during pregnancy using ultrasound, at least in high-income countries. It is well demonstrated in this study that hypoglycemia in the 2nd trimester is associated with SGA at delivery, but is this finding useful to identify the SGA risk during pregnancy in addition to conventional method using ultrasound ?

Response 1
Thank you for the comment. Population-based studies have shown that prenatal identification of SGA babies results in a reduction of adverse perinatal outcomes and stillbirth. However, most SGA babies remain undetected via ultrasound until birth. Therefore, prenatal identification of SGA is very important by using all means. Because we have showed that low GCT results were significantly associated with SGA among pre-gestational underweight women, the additional GCT results on obstetrical management protocols may be helpful to achieve more appropriate recognition of high risk for SGA babies.
We have added a new reference, as well as the following sentence (page 4, lines 6-7): “However, most SGA babies remain undetected via ultrasound until birth.”

Comment 2
Also, if GCT has low reliability that author stated on page 14 line 43, the importance of this finding in this study may be low. Please explain why the estimation of SGA infant using GCT in the 2nd trimester is important.

Response 2
Thank you for the comment. The low reliability of GCT is a limitation in this study. However, according to the present and previous studies [9, 15, 24], low GCT results are useful to predict SGA and perinatal adverse outcomes, although the reliability of GCT results is low. Moreover, we found that the results in underweight women were remarkable.

We have deleted the following sentences (page 14, lines 7-16): “The identification of risk factors for SGA is widely discussed in the literature. Similar to the present study, high insulin sensitivity has been associated with SGA.[2,7–8] However, the combination of GCT results and insulin sensitivity is difficult because the former may have low reliability and may be affected by many factors, such as age, body weight, living environment, change in life partner and situation in which the meal was consumed.[9] This study suggests that low GCT results were significantly associated with SGA among pre-gestational underweight women, and further investigation is necessary to apply low GCT results as a risk factor for SGA. For example, if insulin sensitivity could be measured on the same day of GCT examination, further evidence may be obtained for the relationship between low GCT results and SGA.”
Moreover, we have added the following sentence in the limitation (page 15, lines 16-24; page 16, lines 1 and 2): “Finally, although insulin sensitivity might be associated with SGA, using GCT result as a proxy indicator of insulin sensitivity might be difficult. This is because the former may have low reliability and may be affected by many factors, such as age, body weight, living environment, change in life partner and situation in which the meal was consumed.[9] According to previous studies [9, 15, 24], low GCT results are useful to predict SGA and perinatal adverse outcomes. This study suggests

that low GCT results were significantly associated with SGA among pre-gestational underweight women. In the future, further investigation is necessary to apply low GCT results as a risk factor for SGA. For example, if insulin sensitivity could be measured on the same day of GCT examination, further evidence may be obtained for the relationship between low GCT results and SGA.

Comment 3
FGR and SGA is not identical. Please clarify the usage of these words in the manuscript.

Response 3
Thank you for the comment. Foetal growth restriction (FGR) is not synonymous with SGA. In this manuscript, FGR is used to describe a foetus before birth and SGA is used to describe an infant after birth. Therefore, we have changed FGR to SGA on page 3, line 19; page 4, lines 8 and 9; page 6, line 20; and page 15, line 12 and have added “Risk factors for small-for-gestational-age infants” as a new reference.

Comment 4
a study with two hospital could be called as “multi-centre study”?

Response 4
Thank you for the comment. We have changed the title as follows: “Relationship between maternal hypoglycaemia and small-for-gestational-age infants according to maternal weight status: a retrospective cohort study in two hospitals”.

Comment 5
The first sentence is not similar to what is described in the title. Please check.

Response 5
Thank you for the comment. We have changed the sentence as follows (page 4, line 2): “The relationship between pre-pregnancy body mass index (BMI) and low glucose challenge test (GCT) results by maternal weight status has not been examined.”

Comment 6
Is it reasonable to analyze to combine preterm SGA and term SGA?

Response 6
Thank you for the comment. In this study, we wanted to determine whether low GCT result was significantly associated with SGA births throughout the entire pregnancy period. Therefore, we have combined preterm SGA and term SGA and analysed them together.

Comment 7 and Comment 9
Maternal weight gain was used as a confounding factor, but it is dependent on gestational weeks. Moreover, desired maternal weight gain is also depend on pre-pregnancy weight. If you insist on using maternal weight gain as a variable, you should use weight gain per week like reference 16 instead of gross maternal weight gain. However, it is recommended to consider again whether maternal weight gain is appropriate for a confounder.

Maternal height could be added as a confounder even though pre-pregnancy maternal BMI is put into the model.

Responses 7 and 9
Thank you for the comments. We have eliminated weight gain, added maternal height into the model, and conducted an analysis. For the analysis, please refer to Table 2.

Comment 8
On page 6 line 41-48, authors described confounding variables by citing reference 5, 15, and 16. However, references 5 and 15 are studies among Japanese women. How about other studies in the U.S. and European countries? Reference 6 is a study in the U.S. among women less than 25 years old but the variables used are not identical to this study.

Response 8
Thank you for the comment. We have deleted reference 16 and added the following references:

• Chiavaroli V, Castorani V, Guidone P, et al. Incidence of infants born small- and large-for-gestational-age in an Italian cohort over a 20-year period and associated risk factors. Ital J Pediatr 2016;26:42. doi: 10.1186/s13052-016-0254-7.
• León G, Murcia M, Rebagliato M, et al. Maternal thyroid dysfunction during gestation, preterm delivery, and birthweight. The Infancia y Medio Ambiente Cohort, Spain. Paediatr Perinat Epidemiol 2015;29:113–22.
• Hinkle SN, Albert PS, Mendola P, et al. Differences in risk factors for incident and recurrent small-for-gestational-age birthweight: a hospital-based cohort study. BJOG 2014;121:1080–8.
• Fraser AM, Brockert JE, Ward RH. Association of young maternal age with adverse reproductive outcomes. N Engl J Med 1995;332:1113–7.
• Kozuki N, Katz J, Lee AC, et al. Short maternal stature increases risk of small-for-gestational-age and preterm births in low- and middle-income countries: individual participant data meta-analysis and population attributable fraction. J Nutr 2015;145:2542–50.

Comment 10
In Table 2, the outcome variable (low GCT) should be put in the first place.

Response 10
Thank you for the comment. We have changed Table 2 according to your comment.

Comment 11
The generalized word “hypoglycemia” could be used not only in the title but also in discussion

Response 11
Thank you for the comment. We have changed “low maternal serum glucose levels” to “hypoglycemia” on page 14, lines 6-7.

Comment 12
Please explain whether smoking or other unused variables described in the limitation section is associated with the result of this study and insulin sensitivity/ resistance.

Response 12
In this study, we could not obtain the following data: prior history of childbirth, intake of alcohol and caffeine, anti-phospholipid syndrome, maternal smoking status, kidney disease and inflammatory bowel disease. Some women with SGA in this study may be affected by the above-mentioned risk factors.
The relation between the above-mentioned risk factors and insulin sensitivity is unclear in this study. However, intake of alcohol and caffeine, smoking, kidney disease and inflammatory bowel disease

have been reported to affect insulin sensitivity. Therefore, we have added the following references:

• Boggs DA, Rosenberg L, Ruiz-Narvaez EA, et al. Coffee, tea, and alcohol intake in relation to risk of type 2 diabetes in African American women. Am J Clin Nutr 2010;92:960–6.
• Koppe L, Pelletier CC, Alix PM, et al. Insulin resistance in chronic kidney disease: new lessons from experimental models. Nephrol Dial Transplant 2014;29:1666–74.
• Capristo E, Mingrone G, Addolorato G, et al. Glucose metabolism and insulin sensitivity in inactive inflammatory bowel disease. Aliment Pharmacol Ther 1999;13:209–17.

Moreover, we have revised the sentence (page 15, lines 11-14), “First, data regarding prior history of childbirth, intake of alcohol and caffeine, anti-phospholipid syndrome, maternal smoking status, kidney disease and inflammatory bowel disease were not considered in this study, although these are potential contributors for FGR.” to “First, data regarding prior history of childbirth, intake of alcohol and caffeine, anti-phospholipid syndrome, maternal smoking status, kidney disease, and inflammatory bowel disease, which may affect insulin sensitivity, were not considered in this study, although these are potential contributors for SGA.” We have also added the sentence, “Some women with SGA in this study may be affected by the above-mentioned risk factors.”

Comment 13
In the background, authors hypothesize the insulin resistance is developed late at pregnancy determined by the pre-pregnancy BMI, but in the discussion, authors discuss high insulin sensitivity at early pregnancy.

Response 13
Thank you for the comment. We have deleted the following sentence (page 13, lines 10-12): “Ong et al. suggested that foetal growth may be determined in very early pregnancy and the differences in foetal growth may reveal maternal periconceptual nutrition.”

RESPONSE TO REVIEWER 2

Comment 1
In this study the authors observed that the association between maternal plasma glucose concentrations, one hour after an oral 50 g glucose load, during late pregnancy and the incidence of SGA offspring, appears to be entirely confined to underweight women. The manuscript adds very little to the authors’ original observation (citation [8] in the manuscript). Although the manuscript analyses results from a very slightly larger cohort the results are very similar and the ‘new’ data relates to the evaluation of maternal BMI. Although much is made about the size of the study, because of the sub-analyses the study is probably underpowered. For examples, in Table 2 with maternal BMI <18.5 there were only 7 women with thyroid disease and 8 women with PIH, for women with BMI > 25 there were only 2 teenage pregnancies and 4 women with thyroid disease. These numbers are too small to draw meaningful conclusions.

Response 1
Thank you for the comment. Because this study was a retrospective study, increasing the number of patients and performing analysis again are impossible. Therefore, we have added the following sentence (page 16, lines 2-4): “With regard to the sub-analysis according to maternal weight status, this study is probably underpowered. Therefore, we would like to conduct a more large-scale prospective study in the future.”

Comment 2

With regard to the sub-analysis according to maternal weight status, this study was probably underpowered. There are frequent references to ‘insulin resistance’ and ‘foetal growth restriction’ but no data are provided for either variable

Response 2
Thank you for the comment. Foetal growth restriction (FGR) is not synonymous with SGA. We have used FGR inappropriately in this paper. Therefore, we have changed FGR to SGA on page 3, line 19; page 4, lines 8 and 9; page 6, line 20; and page 15, line 12). Moreover, we have added the following sentence in the limitation section (page 15, lines 15-16): “However, there are no data available regarding the insulin sensitivity of the patients in this study.”

Comment 3
It is not clear why pregnancy BMI was not used as a continuous variable in the analysis of the relationship between maternal glucose and birth weight

Response 3
Thank you for the comment. Insulin sensitivity is typically different according to weight status (A, B). Clinically pregnant women are managed by weight status (C). Therefore, we have not used pregnancy BMI as a continuous variable in this analysis.

A) Arnlöv J, Pencina MJ, Nam BH, et al. Relations of insulin sensitivity to longitudinal blood pressure tracking: variations with baseline age, body mass index, and blood pressure. Circulation 2005;112:1719–27.
B) Gonzales MM, Tarumi T, Miles SC, et al. Insulin sensitivity as a mediator of the relationship between BMI and working memory-related brain activation. Obesity (Silver Spring) 2010;18:2131–37.
C) Minakami H, Maeda T, Fujii T, et al. Guidelines for obstetrical practice in Japan: Japan Society of Obstetrics and Gynecology (JSOG) and Japan Association of Obstetricians and Gynecologists (JAOG) 2014 edition. J Obstet Gynaecol Res 2014;40:1469–99.

Comment 4
could maternal age be a further confounder?

Response 4
Thank you for the comment. The relation between maternal age and prenatal outcomes has been reported many times, particularly about teenage pregnancy. Teenage pregnancy is associated with maternal complications, premature birth, low birth weight, perinatal mortality and increased infant mortality (A, B). Therefore, we think that maternal age should be considered as a confounding factor for SGA. In addition, since the association between teenage pregnancy and SGA was previously investigated, we have not used maternal age as a continuous variable in this analysis (C).

A) Mapanga KG. The perils of adolescent pregnancy. World Health 1997;50:16–18.
B) Lao TT, Ho LF. The obstetric implications of teenage pregnancy. Hum Reprod 1997;12:2303–5.
C) Sebastian T, Yadav B, Jeyaseelan L, et al. Small for gestational age births among South Indian women: temporal trend and risk factors from 1996 to 2010. BMC Pregnancy Childbirth 2015;15:7. doi: 10.1186/s12884-015-0440-4.

Comment 5
Finally, the generalisability of the data to non-Japanese populations should be discussed.

Response 5
Thank you for the comment. We have added the following sentence (page 15, lines 21-22): “Third, the generalisability of our findings may be limited by the homogeneity of this cohort, which contained only Japanese women.”

VERSION 2 – REVIEW

REVIEWER	Sachiko Baba Osaka University, Japan
REVIEW RETURNED	15-Oct-2016

GENERAL COMMENTS	After the revision, the authors’ logic for this study got unsound. Importance of this study, which is crucial point for publication, is denied by authors. Hypotheses were based on maternal insulin sensitivity, but it was not measured, and GCT, which was alternatively used as the exposure of this study, was low liability. Thus, this study did not evaluate the hypotheses the authors have made.

REVIEWER	David B. Dunger University of Cambridge, United Kingdom
REVIEW RETURNED	03-Oct-2016

GENERAL COMMENTS	I believe that the revisions have improved the manuscript. The findings are of interest, although they still do not add very much more to what was found in reference [9].