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Delayed impact of very low birth weight on glucose regulation: observational study

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ABSTRACT

Background: In recent decades, neonatal intensive care has been progressing dramatically and contributed to the improvement in survival rate of very low birth weight (VLBW; <1500g) infants. Although a lot of the first generation of VLBW infants are currently in their twenties or thirties, glucose regulation in their young adulthood is still uncertain.

Objectives: To investigate delayed impact of VLBW on glucose regulation in young adults.

Design: A cross-sectional, observational study included 111 young adults (42 men and 69 women; aged 19-30 years) born with VLBW between 1980 and 1990. Participants underwent standard 75-g oral glucose tolerance test (OGTT). Glucose and insulin levels during OGTT, the pancreatic beta cell function (insulinogenic index and homeostasis model of assessment for beta cell [HOMA-β]), and insulin resistance (homeostasis model of assessment for insulin resistance [HOMA-IR]) were evaluated.

Results: Of 111 young adults with VLBW, 21 subjects (19%) had glucose intolerance: 1 type 2 diabetes; 6 impaired glucose tolerance (IGT);1 impaired fasting glycaemia (IFG); 13 non-diabetes/IGT/IFG with elevated 1-hour glucose levels (>8.6 mmol/l). In logistic regression analysis, male gender was an independent risk factor associated with glucose

intolerance (OR: 3.34; 95%CI: 1.08-10.3; p=0.036). Men had higher levels of glucose (p<0.001) and lower levels of insulin (p=0.005) than women during OGTT. Pancreatic beta cell function was lower in men (insulinogenic index: p=0.002; HOMA-β: p=0.001), although no gender difference was found in insulin resistance (HOMA-IR: p=0.48). In male subjects, logistic regression analysis showed that small for gestational age (SGA) was an independent risk factor associated with glucose intolerance (OR: 33.3; 95%CI: 1.67-662.6; p=0.022).

Conclusions: 19% of individuals with VLBW already had glucose intolerance in young adulthood, and male gender was a significant independent risk factor of glucose intolerance. In male young adults with VLBW, SGA was associated with glucose intolerance.

ARTICLE SUMMARY

Article focus

- Neonatal intensive care has improved the survival rate for very low birth weight infants (VLBW; birth weight <1500g) in recent decades, and the first generation of VLBW infants have only recently reached young adulthood.
- Previous studies have shown the influence of low birth weight (birth weight <2500g) on type 2 diabetes and cardiovascular disease in later life**.**
- However, the glucose regulation in young adults with VLBW (<1500g) is still uncertain.

Key messages

- Of 111 young adults with VLBW in Asian population, 19% of individuals already had glucose intolerance.
- Male gender was a significant independent risk factor of glucose intolerance in young adults with VLBW.
- Small for gestational age (SGA) was associated with glucose intolerance particularly in male young adults with VLBW.

Strengths and limitations of this study

- This is a first study assessing the glucose regulation in young adults with VLBW in Asian population.
- Our study includes a relatively large number of young adults with VLBW.
- We could not obtain information regarding the postnatal growth pattern, which had been shown to be associated with later glucose intolerance in previous studies.

INTRODUCTION

In recent decades, progression of neonatal intensive care has dramatically increased the survival rate of very low birth weight (VLBW; birth weight <1500g) infants worldwide.[1] A lot of them have grown up into young adult (now in their twenties or thirties). To date, epidemiological studies have shown an association between low birth weight and type 2 diabetes and cardiovascular disease in later life.[2-4] Fetal malnutrition in the gestational period, which prevents appropriate fetal growth in utero, is thought to provoke thrifty phenotype in premature babies. This phenotype is assumed to predispose them to subsequent metabolic disorders. For this reason, to foresee the later risk of type 2 diabetes is very crucial for VLBW infants. If prediction is possible, it would lead to early intervention in their lifestyle and subsequently contribute to prevention of type 2 diabetes.[5-7]

However, only a few studies have examined the association between VLBW and glucose regulation in young adulthood[8] because the first generation of VLBW infants have only recently reached young adulthood. To clarify the delayed impact of VLBW on glucose regulation, we investigated glucose regulation in 111 young adults with VLBW by performing detailed oral glucose tolerance test (OGTT), which is useful for evaluation of early signs of glucose intolerance.

METHODS

Study Participants

The birth record database of Seirei Hamamatsu General Hospital (Hamamatsu, Japan) showed that 628 subjects were born with VLBW between 1980 and 1990, and were treated at a neonatal intensive care unit (**Figure 1**). VLBW infants were defined according to the World Health Organization (WHO) criteria: babies whose birth weight was less than 1500g. Out of the 628 subjects, 229 were excluded because of death or severe neurodevelopmental impairment. To the remaining 399 subjects, we sent letters which provided information regarding the study and requested their participation. Among the 399 letters, 98 were returned marked as address unknown (i.e. the remaining 301 letters were thought to reach their destinations). Consequently, 111 subjects (aged 19-30 years) participated in the present study. All participants were Japanese. Small for gestational age (SGA) status was determined according to standards by a study group of the Health Ministry in Japan: a birth weight below the 10th percentile for gestational age. The basal characteristics of participants at birth are summarized in **Table 1**.

Measurements

All participants underwent a standard 75-g OGTT after a ten-hour overnight fast.

Plasma glucose and serum insulin concentrations were examined at 0, 30, 60, 90, 120, and 180 minutes during OGTT. Fasting glucose levels and 2-hour glucose levels were used for diagnosing diabetes mellitus, impaired glucose tolerance (IGT), and impaired fasting glycaemia (IFG) according to the WHO criteria. Since it has been shown that 1-hour plasma glucose concentration is associated with future risk of type 2 diabetes and atherosclerosis, 1-hour plasma glucose above 8.6 mmol/l (155 mg/dl) was included in glucose intolerance.[9 10] Reactive hypoglycemia during OGTT was defined as the level of plasma glucose less than 3.8 mmol/l which causes the response of counter-regulatory hormone release.[11]

We measured plasma glucose and serum insulin levels during OGTT with an autoanalyzer JCA-BM2250 (JEOL, Tokyo, Japan). Plasma glucose was measured by means of hexokinase method. The concentration of serum insulin was measured with chemiluminescent enzyme immunoassay (CLIA). Fasting blood samples were also drawn for other measurements; total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglyceride, and creatinine. Glycated haemoglobin A1c (HbA1c) was measured with high-performance liquid chromatography (HPLC) method using an automated glycohaemoglobin analyzer HLC-723G8 (Tosoh Bioscience, Tokyo, Japan). The values for HbA1c were converted

from Japanese Diabetes Society (JDS) values into National Glycohaemoglobin Standardization Program (NGSP) equivalent values. NGSP equivalent values were calculated with the formula: HbA1c (%) = JDS value (%) +0.4 (http://www.jds.or.jp).

Calculations and Statistical analysis

Pancreatic beta cell function was evaluated by both insulinogenic index and homeostasis model of assessment for beta cell (HOMA-β).[12] Insulinogenic index, the index of early-phase insulin secretion, was calculated as the ratio of the increment in insulin concentration to the increment in glucose concentration ([30-min insulin (μU/ml)–fasting insulin]/[30-min glucose (mmol/l) – fasting glucose]). HOMA-β was calculated as follows: 20 × fasting insulin (μU/ml) / [fasting glucose (mmol/l) –3.5]. Insulin resistance was estimated by homeostasis model of assessment for insulin resistance (HOMA-IR): fasting insulin (μU/ml) × fasting glucose (mmol/l)/22.5.[12] The total amounts of glucose and insulin levels during OGTT were assessed by calculating areas under the curve (AUC) with trapezoid rules. The estimated glomerular filtration rate (eGFR) was calculated according to the Modification of Diet in Renal Disease (MDRD) study equation.

Quantitative variables were expressed as mean and standard deviation (SD) or 95%

confidence interval (CI); categorical variables were presented as number and percentage. Differences between groups were compared using the Student's t-test, the Mann–Whitney U test, the Pearson's χ^2 test, or the repeated measure analysis of variance (ANOVA) as appropriate. A p-value of less than 0.05 was defined as statistically significant. All analyses were conducted using SAS software version 9.2 (SAS Institute, Cary, North Carolina, USA) and the statistical software R version 2.12.2 (http://www.r-project.org).

RESULTS

The basic characteristics of participants at study assessment are shown in **Table 1**. Of 111 young adults with VLBW, 21 subjects (19%) had glucose intolerance: one had type 2 diabetes; six had impaired glucose tolerance (IGT); one had impaired fasting glycaemia (IFG); 13 non-diabetes/IGT/IFG subjects had elevated 1-hour glucose levels (>8.6 mmol/l). Glucose intolerance was more frequent in men than women (26.2 % for men vs 14.5 % for women). In the logistic regression analysis adjusted for family history of diabetes within the second degree, BMI, gestational age, birth weight and SGA/AGA (appropriate for gestational age), male gender was a statistically significant independent factor associated with glucose intolerance (Odds ratio: 3.34; 95% CI:

1.08-10.3; p= 0.036). BMI at study assessment was also a significant independent risk factor of glucose intolerance (**Table 2**).

Gender difference

As male gender was a significant independent risk factor of glucose intolerance, we evaluated the differences in glucose regulation between men and women in the sample group. **Figure 2** shows the glucose and insulin response during OGTT in men and women. Men had significantly higher levels of glucose and lower levels of insulin during OGTT than women by repeated measure ANOVA. $Glucose_{AUC}$ during OGTT also tended to be higher in men (**Table 3**). As for the function of insulin secretion, insulinogenic index and HOMA-ß were significantly lower in men than in women. $Insulin_{AUC}$ also showed a tendency to be lower in men. Reactive hypoglycemia during OGTT tended to be frequent in men. The differences in the mean values of HbA1c and HOMA-IR were not statistically significant. There were no significant gender differences in gestational age (p=0.15), birth weight (p=0.17), age at study assessment (p=0.85), BMI (p=0.88), the proportion of SGA (p=0.76), and that of family history of diabetes within the second degree (p=0.52). The variables for glucose metabolism in men and women are summarized in **Table 3.**

We evaluated the associations between gender and the variables of glucose metabolism by multiple linear regression analysis. Adjustments were made for family history of diabetes within the second degree, BMI, gestational age, birth weight, and SGA/AGA (variables were logarithmically transformed before analysis). In this analysis, male gender had inverse associations with HOMA-β (β: -0.336; 95% CI: -0.509 to -0.163; p< 0.001) and insulinogenic index (β: -0.195; 95% CI: -0.344 to -0.047; p=0.01). $Glucose_{AUC}$ during OGTT tended to be positively associated with male gender (β: 0.056; 95% CI: -0.0047 to 0.116; p=0.071).

In male subjects, after an adjustment for family history of diabetes, BMI, gestational age, and birth weight, the logistic regression analysis showed that SGA was a statistically significant independent factor associated with glucose intolerance (Odds ratio: 33.3; 95% CI: 1.67-662.6; p=0.022).

DISCUSSION

To the best of our knowledge, this is a first study assessing the glucose regulation in young adults with VLBW in Asian population. Our study has indicated that 19% of young adults with VLBW already had glucose intolerance: type 2 diabetes; IGT; IFG; and non-diabetes/IGT/IFG with high 1-hour plasma glucose level. A report from the

Japanese Ministry of Health, Welfare, and Labour in 2007 showed that of 204 general young adults (aged 20-29 years), 2 individuals (0.98%) had high levels of HbA1c (more than 6.0%; NGSP equivalent values), while 3.6% of young adults with VLBW had the HbA1c values more than 6.0% in the present study. In a previous study, Hovi et al. reported that VLBW infants in young adulthood had higher indexes of glucose intolerance compared with term infants.[8] On the other hand, a recent study in the Netherlands showed that preterm birth was not associated with reduced insulin sensitivity in young adulthood.[13] Even though the glucose regulation of premature infants in young adulthood is still controversial, our findings support the standpoint of high prevalence of glucose intolerance in premature infants in young adulthood.

We have also found that male subjects had higher glucose levels during OGTT than females. Previous studies in the general population showed that women had higher postload glucose levels than men, which were explained by differences in body size.[14 15] During standard 75-g OGTT, men and women take the same amount of glucose, which is thought to be high dosage for women relative to their body size. In our study, however, men had significantly higher levels of both fasting and postload glucose concentrations during OGTT. Moreover, male gender was associated with lower beta cell function and the risk of glucose intolerance. These findings might indicate that men

with VLBW are more predisposed to diabetes than women; indeed, recent studies have shown that male premature infants are more vulnerable than females.[16-21] In particular, the male sex is associated with various adverse outcomes including death,[19 21] respiratory dysfunction,[16] intraventricular hemorrhage,[18] autism spectrum,[17] and neurodevelopmental impairment.[20] Interestingly, in the present study, the mean value of height (155cm) in women with VLBW is close to the average value of the Japanese female population (158cm), whereas men with VLBW (164cm) were found to be shorter compared to the Japanese male population (171cm) (average height data of Japanese population were drawn from the report by the Ministry of Education, Culture, Sports, Science, and Technology in Japan). In a previous study, young adults who had been born SGA were shorter and had higher glucose levels than those with a normal birth weight.[22] Reduced final height might be long term consequences of intrauterine retardation, which would also influence glucose regulation. Further investigation is needed to clarify whether the influence of VLBW on physical growth is more remarkable in male than female infants, and elucidate the relationship between physical growth and glucose regulation.

Previous studies have shown that SGA itself is associated with glucose intolerance.[23] In our study, SGA was not significantly associated with glucose intolerance in total

subjects, but was associated in male subjects. The influence of SGA on glucose regulation in young adults with VLBW might be stronger in male than females. It is possible that this gender difference in glucose regulation is owing to the gender difference in the strength of SGA effect.

Strength and weakness of the study

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%). Our study includes a relatively large number of young adults with VLBW, and the individuals with profound complications were carefully excluded in the recruiting process. By this cautious selection, 111 participants would represent the VLBW population spending common life without disability in the general public, and it should make the findings of the study more meaningful. In addition, as participants in the cohort were all Japanese, their racial homogeneity made considerations of ethnic differences in glucose regulation unnecessary. To date, the glucose regulation of Asian young adults with VLBW has been uncertain. Our findings would be useful to clinicians and researchers, and stimulate future large-scale prospective cohort studies in Asian population.

In the present study, we could not obtain information regarding the growth rate in

childhood of all participants. This is a major limitation of the study. The postnatal growth pattern in infancy has been shown to be associated with later glucose intolerance.[24-27] The clinical records of participants were written 20 to 30 years ago, and some of them were no longer preserved. Additionally, maternal factors such as advanced age, smoking, gestational diabetes, and perinatal complications were not available in the present study. These factors might have affected fetal malnutrition and subsequently led to VLBW.

Future Perspective

As neonatal intensive care is making steady progress, an increasing number of young adults with VLBW worldwide will face a greater variety of health problems. Clinician should be aware of the risk of glucose intolerance in young adults with VLBW, and follow up them for a longer period of time. It may be worthwhile for them to check their glucose metabolism with OGTT in their twenties or thirties, which would lead to early intervention in their lifestyle and subsequently contribute to prevention of type 2 diabetes and cardiovascular disease.[5-7] In the present study, we have found that male gender was a significant independent risk factor of glucose intolerance in young adults with VLBW. In addition to the gender difference, future studies are required to focus on

the factors affecting the glucose metabolism in VLBW infants.

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Contributors: RS and HW drafted the paper. RS conceived the study. RS and KS developed the idea. RS, HW, KS, SO, RG, MM, and HN were involved in study design. RS and KS participated in data acquisition. RS, HW, HM, EI, MT, and MM were engaged in data analysis.

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Competing Interest: None.

Ethical approval: The study was approved by the ethical committee of Seirei Hamamatsu General Hospital. All participants provided written informed consent to participate in the study.

Data sharing statement: Data will not be publically accessible. However, interested individuals may contact the authors.

REFERENCES

1. Field DJ, Dorling JS, Manktelow BN, Draper ES. Survival of extremely premature babies in a geographically defined population: prospective cohort study of 1994-9 compared with 2000-5. *BMJ* 2008;336(7655):1221-3.
2. Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS. Fetal nutrition and cardiovascular disease in adult life. *Lancet* 1993;341(8850):938-41.
3. Kaijser M, Bonamy AK, Akre O, Cnattingius S, Granath F, Norman M, et al. Perinatal risk factors for diabetes in later life. *Diabetes* 2009;58(3):523-6.
4. Whincup PH, Kaye SJ, Owen CG, Huxley R, Cook DG, Anazawa S, et al. Birth weight and risk of type 2 diabetes: a systematic review. *JAMA* 2008;300(24):2886-97.
5. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* 2002;346(6):393-403.
6. Eriksson KF, Lindgarde F. Prevention of type 2 (non-insulin-dependent) diabetes mellitus by diet and physical exercise. The 6-year Malmo feasibility study. *Diabetologia* 1991;34(12):891-8.
7. Tuomilehto J, Lindstrom J, Eriksson JG, Valle TT, Hamalainen H, Ilanne-Parikka P, et al. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N Engl J Med* 2001;344(18):1343-50.
8. Hovi P, Andersson S, Eriksson JG, Jarvenpaa AL, Strang-Karlsson S, Makitie O, et al. Glucose regulation in young adults with very low birth weight. *N Engl J Med* 2007;356(20):2053-63.
9. Abdul-Ghani MA, Abdul-Ghani T, Ali N, Defronzo RA. One-hour plasma glucose concentration and the metabolic syndrome identify subjects at high risk for future type 2 diabetes. *Diabetes Care* 2008;31(8):1650-5.
10. Succurro E, Marini MA, Arturi F, Grembiale A, Lugara M, Andreozzi F, et al. Elevated one-hour post-load plasma glucose levels identifies subjects with normal glucose tolerance but early carotid atherosclerosis. *Atherosclerosis* 2009;207(1):245-9.
11. Zammitt NN, Frier BM. Hypoglycemia in type 2 diabetes: pathophysiology, frequency, and effects of different treatment modalities. *Diabetes Care* 2005;28(12):2948-61.
12. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC.

Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28(7):412-9.

13. Willemsen RH, Leunissen RW, Stijnen T, Hokken-Koelega AC. Prematurity is not associated with reduced insulin sensitivity in adulthood. *J Clin Endocrinol Metab* 2009;94(5):1695-700.
14. Faerch K, Borch-Johnsen K, Vaag A, Jorgensen T, Witte DR. Sex differences in glucose levels: a consequence of physiology or methodological convenience? The Inter99 study. *Diabetologia* 2010;53(5):858-65.
15. Sicree RA, Zimmet PZ, Dunstan DW, Cameron AJ, Welborn TA, Shaw JE. Differences in height explain gender differences in the response to the oral glucose tolerance test- the AusDiab study. *Diabet Med* 2008;25(3):296-302.
16. Henderson-Smart DJ, Hutchinson JL, Donoghue DA, Evans NJ, Simpson JM, Wright I. Prenatal predictors of chronic lung disease in very preterm infants. *Arch Dis Child Fetal Neonatal Ed* 2006;91(1):F40-5.
17. Johnson S, Hollis C, Kochhar P, Hennessy E, Wolke D, Marlow N. Autism spectrum disorders in extremely preterm children. *J Pediatr* 2010;156(4):525-31 e2.
18. Mohamed MA, Aly H. Male gender is associated with intraventricular hemorrhage. *Pediatrics* 2010;125(2):e333-9.
19. Morse SB, Wu SS, Ma C, Ariet M, Resnick M, Roth J. Racial and gender differences in the viability of extremely low birth weight infants: a population-based study. *Pediatrics* 2006;117(1):e106-12.
20. Spinillo A, Montanari L, Gardella B, Roccio M, Stronati M, Fazzi E. Infant sex, obstetric risk factors, and 2-year neurodevelopmental outcome among preterm infants. *Dev Med Child Neurol* 2009;51(7):518-25.
21. Tyson JE, Parikh NA, Langer J, Green C, Higgins RD. Intensive care for extreme prematurity--moving beyond gestational age. *N Engl J Med* 2008;358(16):1672-81.
22. Leger J, Levy-Marchal C, Bloch J, Pinet A, Chevenne D, Porquet D, et al. Reduced final height and indications for insulin resistance in 20 year olds born small for gestational age: regional cohort study. *BMJ* 1997;315(7104):341-7.
23. Saenger P, Czernichow P, Hughes I, Reiter EO. Small for gestational age: short stature and beyond. *Endocr Rev* 2007;28(2):219-51.
24. Crowther NJ, Cameron N, Trusler J, Toman M, Norris SA, Gray IP. Influence of catch-up growth on glucose tolerance and beta-cell function in 7-year-old children: results from the birth to twenty study. *Pediatrics*

2008;121(6):e1715-22.

25. Eriksson JG, Osmond C, Kajantie E, Forsen TJ, Barker DJ. Patterns of growth among children who later develop type 2 diabetes or its risk factors. *Diabetologia* 2006;49(12):2853-8.
26. Finken MJ, Keijzer-Veen MG, Dekker FW, Frolich M, Hille ET, Romijn JA, et al. Preterm birth and later insulin resistance: effects of birth weight and postnatal growth in a population based longitudinal study from birth into adult life. *Diabetologia* 2006;49(3):478-85.
27. Leunissen RW, Kerkhof GF, Stijnen T, Hokken-Koelega A. Timing and tempo of first-year rapid growth in relation to cardiovascular and metabolic risk profile in early adulthood. *JAMA* 2009;301(21):2234-42.

Table 1. Clinical characteristics of young adults with very low birth weight (VLBW) at birth and at study assessment

	Total (n=111)	Men (n=42)	Women (n=69)
At birth			
Gestational Age (wk)	29.7 (3.1)	29.2 (3.1)	30.0 (3.2)
Weight (g)	1152 (235)	1116 (233)	1174 (235)
ELBW	33 (29.7)	16 (38.1)	17 (24.6)
SGA*	39 (35.1)	14 (33.3)	25 (36.2)
Birth from multiple pregnancy	18 (16.2)	9 (21.4)	9 (13.0)
At study assessment			
Age (yr)	24.8 (3.0)	24.7 (3.4)	24.9 (2.9)
Family history of diabetes	22 (19.8)	7 (16.7)	15 (21.7)
Height (m)	1.58 (0.07)	1.64 (0.06)	1.55 (0.06)
Body Weight (kg)	52.3 (10.1)	54.9 (8.8)	50.7 (10.5)
BMI (kg/m^2)	20.9 (3.8)	20.5 (2.9)	21.1 (4.2)
Blood pressure (mmHg)			
Systolic	118 (16)	121 (17)	116 (14)
Diastolic	70 (11)	69 (12)	70 (10)
Cholesterol (mg/dl)			
Total	184.8 (31.3)	192.5 (38.0)	180.1 (25.7)
Low-density lipoprotein	104.3 (28.4)	112.1 (35.5)	99.5 (22)
High- density lipoprotein	65.7 (13.3)	64.1 (13.9)	66.6 (13.0)
Triglycerides (mg/dl)	81.8 (72.2)	96.2 (106.7)	73.0 (36.9)
Renal function			
Creatinine (mg/dl)	0.66 (0.13)	0.79 (0.11)	0.58 (0.81)
eGFR (ml/min/1.73 m^2)†	104.6 (18.4)	103.0 (17.7)	105.6 (18.9)

ELBW=Extremely low birth weight (<1000g); SGA=Small for gestational age; BMI=body mass index; eGFR=estimated glomerular filtration rate.

Data are expressed as mean (SD) or number (%).

* Determined by a birth weight below the 10th percentile for gestational age according to standards defined by a study group of the Health Ministry in Japan.

†Calculated according to the Modification of Diet in Renal Disease (MDRD) study equation.

Table 2. Correlated factors for glucose intolerance* in young adults with very low birth weight (VLBW) assessed by logistic regression analyses

Variable	Odds Ratio	95% CI	P-value
Gender (Male)	3.34	1.08 - 10.3	0.036
Factors at birth			
Gestational Age (wk)	0.77	0.53 - 1.12	0.165
Weight (g)	1.00	1.00 - 1.01	0.085
SGA	2.56	0.37 - 17.5	0.340
Factors at study assessment			
Family history of diabetes	1.92	0.49 - 7.57	0.353
BMI (kg/m^2)	1.29	1.11 - 1.49	0.001

SGA=Small for gestational age; BMI=body mass index.

*Glucose intolerance includes Diabetes, IGT (impaired glucose tolerance), IFG (impaired fasting glycaemia), and Non-diabetes/IGT/IFG with elevated 1-hour glucose levels (> 8.6mmol/l).

Table 3. Gender differences in glucose regulation in young adults with very low birth weight (VLBW)

	Men (n=42)	Women (n=69)	P-value
Glucose intolerance	11 (26.2)	10 (14.5)	0.13
Diabetes	0	1	
IGT	2	4	
IFG	1	0	
Non-diabetes/IGT/IFG with elevated 1-hour glucose levels*	8	5	
HbA1c (%)	5.39 (5.31 - 5.47)	5.39 (5.31 - 5.47)	0.64
HOMA-β	72.5 (59.0 - 86.0)	103 (87.2 - 119.6)	0.001
HOMA-IR	1.4 (1.14 - 1.69)	1.6 (1.29 - 1.90)	0.48
Variable during OGTT			
Insulinogenic Index	1.1 (0.64 - 1.60)	1.4 (1.18 - 1.71)	0.002
$Glucose_{AUC}$ (mmol/l ×hr)	18.8 (17.9 - 19.7)	18.2 (17.3 - 19.0)	0.089
$Insulin_{AUC}$ (μU/ml × hr)	135.3 (98.7 - 171.9)	145.1 (121.7 - 168.6)	0.052
Reactive hypoglycemia	17 (40)	17 (25)	0.079

IGT=impaired glucose tolerance; IFG=impaired fasting glycaemia; HOMA-β=homeostasis model of assessment for beta cell; HOMA-IR=homeostasis model of assessment for insulin resistance.

Data are expressed as mean (95% CI) or number (%).

*Defined as 1-hour glucose levels > 8.6mmol/l.

Figure Legends

Figure 1. Flow of participants through the study

(There is no figure legend in Figure 1.)

Figure 2. The gender differences of glucose and insulin levels during OGTT in young adults with very low birth weight (VLBW)

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).

The numerical data of Figure 2.

Male (n=42)

	0 (min)	30	60	90	120	180
Plasma glucose (mg/dl)	5.208 (0.3695)	8.579 (1.272)	7.167 (1.824)	6.262 (1.523)	5.749 (1.102)	4.360 (1.205)
Insulin (microU/ml)	6.057 (3.519)	64.95 (64.53)	53.70 (47.86)	53.27 (49.61)	49.84 (50.66)	20.94 (24.37)

Female (n=69)

	0 (min)	30	60	90	120	180
Plasma glucose (mg/dl)	4.930 (0.4266)	7.529 (1.460)	6.596 (1.946)	6.178 (1.541)	5.829 (1.487)	4.881 (1.121)
Insulin (microU/ml)	7.090 (5.107)	66.22 (42.62)	57.09 (51.97)	54.95 (44.39)	51.11 (39.85)	31.82 (28.03)

Data are expressed as mean (SD).

Figure 1. Flow of Participants through the study

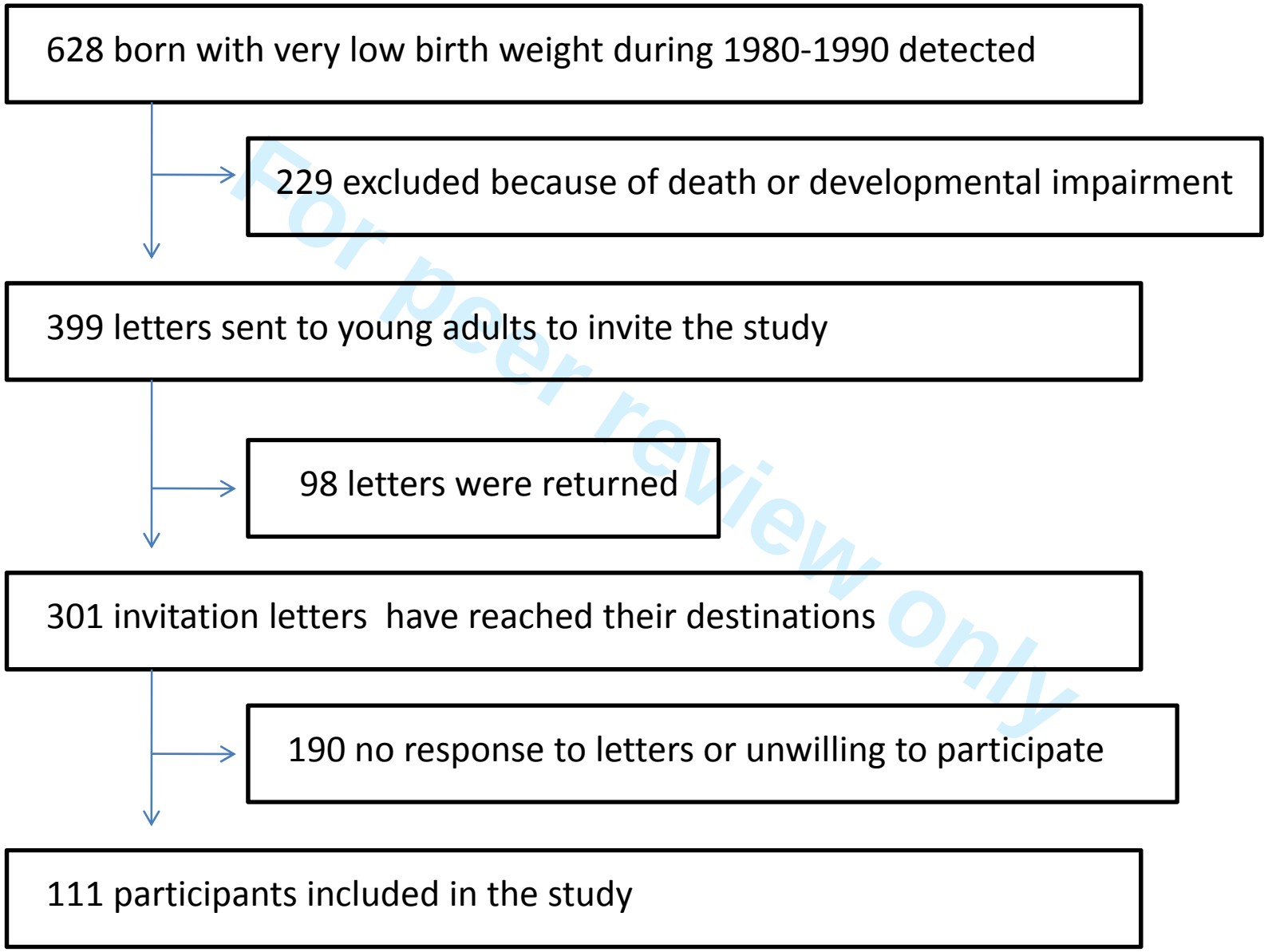
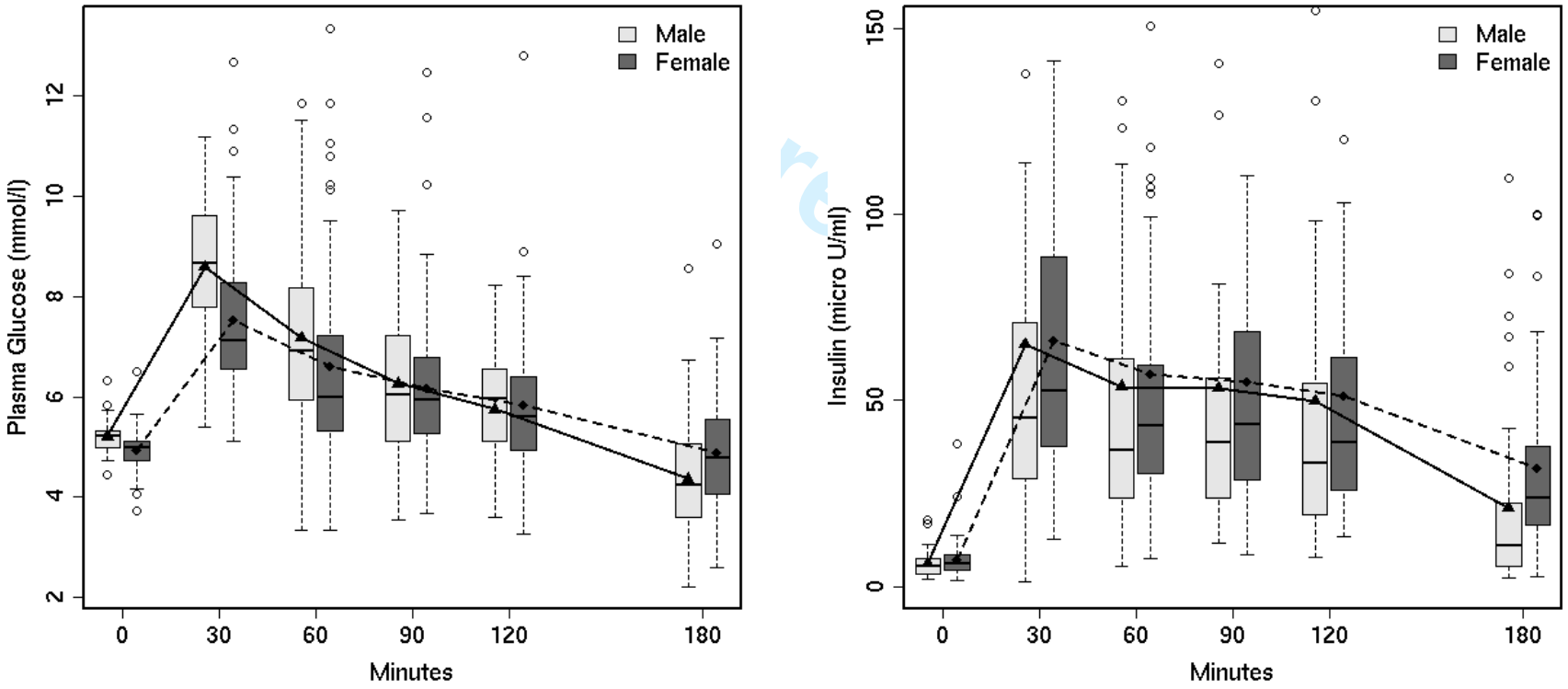


Figure 2. The gender differences of glucose and insulin levels during OGTT in young adults with very low birth weight (VLBW)



STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	7
Objectives	3	State specific objectives, including any prespecified hypotheses	7
Methods			
Study design	4	Present key elements of study design early in the paper	8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	8
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	8
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	9-10
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	n/a
Bias	9	Describe any efforts to address potential sources of bias	*8*
Study size	10	Explain how the study size was arrived at	8
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	10
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	10-11, 13
		(*b*) Describe any methods used to examine subgroups and interactions	13
		(*c*) Explain how missing data were addressed	n/a
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	n/a
		(*e*) Describe any sensitivity analyses	n/a
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	8
		(c) Consider use of a flow diagram	Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1
		(b) Indicate number of participants with missing data for each variable of interest	n/a
Outcome data	15*	Report numbers of outcome events or summary measures	n/a
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	12-13
		(*b*) Report category boundaries when continuous variables were categorized	n/a
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	13
Discussion			
Key results	18	Summarise key results with reference to study objectives	13-14
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	16
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	15-16
Generalisability	21	Discuss the generalisability (external validity) of the study results	16
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	18

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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

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

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Keywords: diabetes mellitus, very low birth weight (VLBW), small for gestational age (SGA), gender difference, oral glucose tolerance test (OGTT)

ABSTRACT

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

Design: Cross-sectional, observational study.

Setting: A general hospital in Hamamatsu, Japan.

Participants: 111 young adults (42 men and 69 women; aged 19-30 years) born with VLBW between 1980 and 1990. Participants underwent standard 75-g oral glucose tolerance test (OGTT).

Primary and secondary outcome measures: The primary outcomes were glucose and insulin levels during OGTT, and risk factors for a category of hyperglycemia defined as follows: diabetes mellitus; impaired glucose tolerance (IGT); impaired fasting glycemia (IFG); and non-diabetes/IGT/IFG with elevated 1-hour glucose levels (>8.6 mmol/l). The secondary outcomes were the pancreatic beta cell function (insulinogenic index and homeostasis model of assessment for beta cell [HOMA-β]), and insulin resistance (homeostasis model of assessment for insulin resistance [HOMA-IR]).

Results: Of 111 young adults with VLBW, 21 subjects (19%) had hyperglycemia: 1 had type 2 diabetes; 6 had IGT; 1 had IFG; and 13 had non-diabetes/IGT/IFG with elevated 1-hour glucose levels. In logistic regression analysis, male gender was an independent

risk factor associated with hyperglycemia (OR: 3.34; 95%CI: 1.08-10.3; p=0.036). 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). Pancreatic beta cell function was lower in men (insulinogenic index: p=0.002; HOMA-β: p=0.001), although no gender difference was found in insulin resistance (HOMA-IR: p=0.477). In male subjects, logistic regression analysis showed that small for gestational age (SGA) was an independent risk factor associated with hyperglycemia (OR: 33.3; 95%CI: 1.67-662.6; p=0.022).

Conclusions: 19% of individuals with VLBW already had hyperglycemia in young adulthood, and male gender was a significant independent risk factor of hyperglycemia. In male young adults with VLBW, SGA was associated with hyperglycemia.

ARTICLE SUMMARY

Article focus

- Neonatal intensive care has improved the survival rate for very low birth weight infants (VLBW; birth weight <1500g) in recent decades, and the first generation of VLBW infants have only recently reached young adulthood.
- 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.
- The present study investigated glucose regulation in young adults with VLBW in an Asian population, and determined the factors associated with hyperglycemia.

Key messages

- Of 111 young adults with VLBW, 19% of individuals already had hyperglycemia (type 2 diabetes, IGT, IFG, and non-diabetes/IGT/IFG with elevated 1-hour glucose levels).
- Male gender was a significant independent risk factor of hyperglycemia in young adults with VLBW.
- Small for gestational age (SGA) was associated with hyperglycemia particularly in

male young adults with VLBW.

Strengths and limitations of this study

- This is the first study assessing the glucose regulation in young adults with VLBW in an Asian population.
- This study does not provide information on postnatal growth patterns, which have been shown to be associated with later hyperglycemia in previous studies.
- The study design with no control subjects makes it impossible to address the delayed impact of VLBW itself on glucose regulation.

INTRODUCTION

In recent decades, progression of neonatal intensive care has dramatically increased the survival rate of very low birth weight (VLBW; birth weight <1500g) infants worldwide.[1] A lot of them have grown up into young adult (now in their twenties or thirties). To date, epidemiological studies have shown an association between low birth weight and type 2 diabetes and cardiovascular disease in later life.[2-4] Fetal malnutrition in the gestational period, which prevents appropriate fetal growth in utero, is thought to provoke thrifty phenotype in premature babies. This phenotype is assumed to predispose them to subsequent metabolic disorders. 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.[5-7]

The first generation of VLBW infants have only recently reached young adulthood. A few studies have shown that VLBW (or preterm) is associated with glucose intolerance in young adulthood in Caucasian populations,[8 9] while the glucose regulation in Asian young adults with VLBW remains uncertain. To clarify the characteristics of glucose metabolism in young adults with VLBW in an Asian population, we investigated glucose regulation in 111 young adults with VLBW by performing detailed oral glucose tolerance test (OGTT), which is useful for evaluation of early signs of glucose

intolerance.

METHODS

Study Participants

The birth record database of Seirei Hamamatsu General Hospital (Hamamatsu, Japan) showed that 628 subjects were born with VLBW between 1980 and 1990, and were treated at a neonatal intensive care unit (**Figure 1**). VLBW infants were defined according to the World Health Organization (WHO) criteria: babies whose birth weight was less than 1500g. Out of the 628 subjects, 229 were excluded because of death (n=132) or severe neurodevelopmental impairment (n=97). To the remaining 399 subjects, we sent letters which provided information regarding the study and requested their participation. Among the 399 letters, 98 were returned marked as address unknown (i.e. the remaining 301 letters were thought to reach their destinations). Consequently, 111 subjects (aged 19-30 years) participated in the present study. All participants were Japanese. Small for gestational age (SGA) status was determined according to standards by a study group of the Health Ministry in Japan: a birth weight below the 10th percentile for gestational age.[10] The basal characteristics of participants at birth are summarized in **Table 1**.

Measurements

All participants underwent a standard 75-g OGTT after a ten-hour overnight fast. Plasma glucose and serum insulin concentrations were examined at 0, 30, 60, 90, 120, and 180 minutes during OGTT. Fasting glucose levels and 2-hour glucose levels were used for diagnosing diabetes mellitus, impaired glucose tolerance (IGT), and impaired fasting glycaemia (IFG) according to the WHO criteria.[11] Since it has been shown that 1-hour plasma glucose concentration is associated with future risk of type 2 diabetes and atherosclerosis, 1-hour plasma glucose above 8.6 mmol/l (155 mg/dl) was included as a category of hyperglycemia.[12 13] Reactive hypoglycemia during OGTT was defined as the level of plasma glucose less than 3.8 mmol/l which causes the response of counter-regulatory hormone release.[14]

We measured plasma glucose and serum insulin levels during OGTT with an autoanalyzer JCA-BM2250 (JEOL, Tokyo, Japan). Plasma glucose was measured by means of hexokinase method. The concentration of serum insulin was measured with chemiluminescent enzyme immunoassay (CLIA). Fasting blood samples were also drawn for other measurements; total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglyceride, and creatinine.

Glycated haemoglobin A1c (HbA1c) was measured with high-performance liquid chromatography (HPLC) method using an automated glycohaemoglobin analyzer HLC-723G8 (Tosoh Bioscience, Tokyo, Japan). The values for HbA1c were converted from Japanese Diabetes Society (JDS) values into National Glycohaemoglobin Standardization Program (NGSP) equivalent values. NGSP equivalent values were calculated with the formula: HbA1c (%) = JDS value (%) +0.4.[15]

Calculations and Statistical analysis

Pancreatic beta cell function was evaluated by both insulinogenic index and homeostasis model of assessment for beta cell (HOMA-β).[16] Insulinogenic index, the index of early-phase insulin secretion, was calculated as the ratio of the increment in insulin concentration to the increment in glucose concentration ([30-min insulin (μU/ml)–fasting insulin]/[30-min glucose (mmol/l) – fasting glucose]). HOMA-β was calculated as follows: 20 × fasting insulin (μU/ml) / [fasting glucose (mmol/l) –3.5]. Insulin resistance was estimated by homeostasis model of assessment for insulin resistance (HOMA-IR): fasting insulin (μU/ml) × fasting glucose (mmol/l)/22.5.[16] The total amounts of glucose and insulin levels during OGTT were assessed by calculating areas under the curve (AUC) with trapezoid rules. The estimated glomerular filtration

rate (eGFR) was calculated according to the following formula, as recommended by the Japanese Society of Nephrology: eGFR (ml/min/1.73m^2) = 194 × Cre$^{-1.094}$ × Age$^{-0.287}$ (× 0.739 if the subject is a woman).[17]

Quantitative variables were expressed as mean and standard deviation (SD) or 95% confidence interval (CI); categorical variables were presented as number and percentage. Differences between groups were compared using the Student's t-test, the Mann–Whitney U test, the Pearson's χ^2 test, or the repeated measure analysis of variance (ANOVA) as appropriate. The data on insulin levels during OGTT were logarithmically transformed before the repeated measure ANOVA. Logistic regression analysis which included gender, family history of diabetes within the second degree, BMI, gestational age, birth weight, and SGA/AGA (appropriate for gestational age) was performed to estimate odds ratios for the category of hyperglycemia. For the further investigation into glucose regulation, multiple linear regression analysis was conducted. Gender, family history of diabetes, BMI, gestational age, birth weight, and SGA/AGA were included in the model. The data on HOMA-β, HOMA-IR, Insulinogenic Index, and $Glucose_{AUC}$ were logarithmically transformed before analysis to meet the assumptions of normality. A p-value of less than 0.05 was defined as statistically significant. All analyses were conducted using SAS software version 9.2 (SAS Institute,

Cary, North Carolina, USA) and the statistical software R version 2.12.2 (http://www.r-project.org).

RESULTS

The basic characteristics of participants at study assessment are shown in **Table 1**. Of 111 young adults with VLBW, 21 subjects (19%) had hyperglycemia: one had type 2 diabetes; six had impaired glucose tolerance (IGT); one had impaired fasting glycaemia (IFG); 13 non-diabetes/IGT/IFG subjects had elevated 1-hour glucose levels (>8.6 mmol/l). Hyperglycemia was more frequent in men than women (26.2 % for men vs 14.5 % for women). In the logistic regression analysis adjusted for family history of diabetes within the second degree, BMI, gestational age, birth weight and SGA/AGA, male gender was a statistically significant independent factor associated with hyperglycemia (Odds ratio: 3.34; 95% CI: 1.08-10.3; p= 0.036). BMI at study assessment was also associated with hyperglycemia (**Table 2**).

Gender difference

As male gender was a significant independent risk factor of hyperglycemia, we evaluated the differences in glucose regulation between men and women in the sample

group. **Figure 2** shows the glucose and insulin response during OGTT in men and women. 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). $Glucose_{AUC}$ during OGTT tended to be higher in male subjects (**Table 3**). As for the function of insulin secretion, insulinogenic index and HOMA-ß were significantly lower in men than in women. $Insulin_{AUC}$ also showed a tendency to be lower in male subjects. Reactive hypoglycemia during OGTT tended to be frequent in men. The differences in the mean values of HbA1c and HOMA-IR were not statistically significant. There were no significant gender differences in gestational age ($p=0.145$), birth weight ($p=0.168$), age at study assessment ($p=0.845$), BMI ($p=0.879$), the proportion of SGA ($p=0.756$), and that of family history of diabetes within the second degree ($p=0.516$). The variables for glucose metabolism in men and women are summarized in **Table 3.**

We evaluated the associations between gender and the variables of glucose metabolism by multiple linear regression analysis. Adjustments were made for family history of diabetes within the second degree, BMI, gestational age, birth weight, and SGA/AGA. In this analysis, male gender had inverse associations with HOMA-β (β: -0.336; 95%

CI: -0.509 to -0.163; p< 0.001) and insulinogenic index (β: -0.195; 95% CI: -0.344 to -0.047; p=0.01). $Glucose_{AUC}$ during OGTT tended to be positively associated with male gender (β: 0.056; 95% CI: -0.0047 to 0.116; p=0.071).

In male subjects, after an adjustment for family history of diabetes, BMI, gestational age, and birth weight, the logistic regression analysis showed that SGA was a statistically significant independent factor associated with hyperglycemia (Odds ratio: 33.3; 95% CI: 1.67-662.6; p=0.022).

DISCUSSION

To the best of our knowledge, this is the first study assessing the glucose regulation in young adults with VLBW in an Asian population. Our study has indicated that 19% of young adults with VLBW already had hyperglycemia: type 2 diabetes; IGT; IFG; and non-diabetes/IGT/IFG with high 1-hour plasma glucose level. A report from the Japanese Ministry of Health, Welfare, and Labour in 2007 showed that of 204 general young adults (aged 20-29 years), 2 individuals (0.98%) had high levels of HbA1c (more than 6.0%; NGSP equivalent values),[18] while 3.6% of young adults with VLBW had the HbA1c values more than 6.0% in the present study. In a previous study, Hovi et al. reported that VLBW infants in young adulthood had higher indexes of glucose

intolerance compared with term infants.[9] A recent epidemiological study has also shown that preterm birth is associated with an increased risk of diabetes in young adults.[8] On the other hand, a study in the Netherlands showed that preterm birth was not associated with reduced insulin sensitivity in young adulthood.[19] The findings of that study may be biased by the way of recruiting the control subjects born at term. Our findings would be in line with the standpoint of high prevalence of hyperglycemia in premature infants in young adulthood, although absence of control subjects presents a limitation of demonstrating the impact of VLBW itself on glucose regulation.

We have also found that male subjects had higher glucose levels during OGTT than females. Previous studies in the general population showed that women had higher postload glucose levels than men, which were explained by differences in body size.[20 21] During standard 75-g OGTT, men and women take the same amount of glucose, which is thought to be high dosage for women relative to their body size. In our study, however, men had significantly higher levels of both fasting and postload glucose concentrations during OGTT. Moreover, male gender was associated with lower beta cell function and the risk of glucose intolerance. These findings might indicate that men with VLBW are more predisposed to diabetes than women; indeed, recent studies have shown that male premature infants are more vulnerable than females.[22-27] In particular,

the male sex is associated with various adverse outcomes including death,[25 27] respiratory dysfunction,[22] intraventricular hemorrhage,[24] autism spectrum,[23] and neurodevelopmental impairment.[26] Interestingly, in the present study, the mean value of height (155cm) in women with VLBW is close to the average value of the Japanese female population (158cm), whereas men with VLBW (164cm) were found to be shorter compared to the Japanese male population (171cm) (average height data of Japanese population were drawn from the report by the Ministry of Education, Culture, Sports, Science, and Technology in Japan).[28] In a previous study, young adults who had been born SGA were shorter and had higher glucose levels than those with a normal birth weight.[29] Reduced final height might be long term consequences of intrauterine retardation, which would also influence glucose regulation. Further investigation is needed to clarify whether the influence of VLBW on physical growth is more remarkable in male than female infants, and elucidate the relationship between physical growth and glucose regulation.

Previous studies have shown that SGA itself is associated with glucose intolerance.[30] In our study, SGA was not significantly associated with hyperglycemia in total subjects, but was associated in male subjects. The influence of SGA on glucose regulation in young adults with VLBW might be stronger in male than females. It is possible that this

gender difference in glucose regulation is owing to the gender difference in the strength of SGA effect.

Strength and weakness of the study

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).[31] Our study includes a relatively large number of young adults with VLBW, and the individuals with profound complications were carefully excluded in the recruiting process. In addition, as participants in the cohort were all Japanese, their racial homogeneity made considerations of ethnic differences in glucose regulation unnecessary. To date, the glucose regulation of Asian young adults with VLBW has been uncertain. Our findings would be useful to clinicians and researchers, and stimulate future large-scale prospective cohort studies in Asian populations.

In the present study, we could not obtain information regarding the growth rate in childhood of all participants. This is a major limitation of the study. The postnatal growth pattern in infancy has been shown to be associated with later glucose intolerance.[32-35] The clinical records of participants were written 20 to 30 years ago, and some of them were no longer preserved. Additionally, maternal factors such as advanced

age, smoking, gestational diabetes, and perinatal complications were not available in the present study. These factors might have affected fetal malnutrition and subsequently led to VLBW. 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. 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.

Future Perspective

As neonatal intensive care is making steady progress, an increasing number of young adults with VLBW worldwide will face a greater variety of health problems. Clinician should be aware of the risk of hyperglycemia in young adults with VLBW, and follow up them for a longer period of time. It may be worthwhile for them to check their glucose metabolism with OGTT in their twenties or thirties, which would lead to early intervention in their lifestyle and subsequently contribute to prevention of type 2 diabetes and cardiovascular disease.[5-7] In the present study, we have found that male gender was a significant independent risk factor of hyperglycemia in young adults with

VLBW. In addition to the gender difference, future studies are required to focus on the factors affecting the glucose metabolism in VLBW infants.

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Contributors: RS and HW drafted the paper. RS conceived the study. RS and KS developed the idea. RS, HW, KS, SO, RG, MM, and HN were involved in study design. RS and KS participated in data acquisition. RS, HW, HM, EI, MT, and MM were engaged in data analysis. All the authors contributed to the interpretation of the findings. All the authors were involved in the revision and approved the final version.

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Competing Interest: None.

Ethical approval: The study was approved by the ethical committee of Seirei Hamamatsu General Hospital. All participants provided written informed consent to participate in the study.

Data sharing statement: Data will not be publically accessible. However, interested individuals may contact the authors.

REFERENCES

1. Field DJ, Dorling JS, Manktelow BN, et al. Survival of extremely premature babies in a geographically defined population: prospective cohort study of 1994-9 compared with 2000-5. *BMJ* 2008;336(7655):1221-3.
2. Barker DJ, Gluckman PD, Godfrey KM,et al. Fetal nutrition and cardiovascular disease in adult life. *Lancet* 1993;341(8850):938-41.
3. Kaijser M, Bonamy AK, Akre O, et al. Perinatal risk factors for diabetes in later life. *Diabetes* 2009;58(3):523-6.
4. Whincup PH, Kaye SJ, Owen CG, et al. Birth weight and risk of type 2 diabetes: a systematic review. *JAMA* 2008;300(24):2886-97.
5. Knowler WC, Barrett-Connor E, Fowler SE, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* 2002;346(6):393-403.
6. Eriksson KF, Lindgarde F. Prevention of type 2 (non-insulin-dependent) diabetes mellitus by diet and physical exercise. The 6-year Malmo feasibility study. *Diabetologia* 1991;34(12):891-8.
7. Tuomilehto J, Lindstrom J, Eriksson JG, et al. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N Engl J Med* 2001;344(18):1343-50.
8. Crump C, Winkleby MA, Sundquist K, et al. Risk of diabetes among young adults born preterm in Sweden. *Diabetes Care* 2011;34(5):1109-13.
9. Hovi P, Andersson S, Eriksson JG, et al. Glucose regulation in young adults with very low birth weight. *N Engl J Med* 2007;356(20):2053-63.
10. The study group of the Health Ministry in Japan. Fetal growth curves of Japanese, 1994. pp 61.
11. World Health Organization. Definition, diagnosis and classification of diabetes mellitus and its complications. Geneva: World Health Organization, 1999.
12. Abdul-Ghani MA, Abdul-Ghani T, Ali N, et al. One-hour plasma glucose concentration and the metabolic syndrome identify subjects at high risk for future type 2 diabetes. *Diabetes Care* 2008;31(8):1650-5.
13. Succurro E, Marini MA, Arturi F, et al. Elevated one-hour post-load plasma glucose levels identifies subjects with normal glucose tolerance but early carotid atherosclerosis. *Atherosclerosis* 2009;207(1):245-9.
14. Zammitt NN, Frier BM. Hypoglycemia in type 2 diabetes: pathophysiology, frequency, and effects of different treatment modalities. *Diabetes Care*

2005;28(12):2948-61.

15. The Committee of Japan Diabetes Society on the diagnostic criteria of diabetes mellitus. Report of the Committee on the classification and diagnostic criteria of diabetes mellitus. *J Japan Diab Soc* 2010; 53 450-67.
16. Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28(7):412-9.
17. Matsuo S, Imai E, Horio M, et al. Revised equations for estimated GFR from serum creatinine in Japan. *Am J Kidney Dis* 2009;53(6):982-92.
18. The Ministry of Health, Labour, and Welfare. The National Health and Nutrition Survey in Japan, 2007. pp 187.
19. Willemsen RH, Leunissen RW, Stijnen T, et al. Prematurity is not associated with reduced insulin sensitivity in adulthood. *J Clin Endocrinol Metab* 2009;94(5):1695-700.
20. Faerch K, Borch-Johnsen K, Vaag A, et al. Sex differences in glucose levels: a consequence of physiology or methodological convenience? The Inter99 study. *Diabetologia* 2010;53(5):858-65.
21. Sicree RA, Zimmet PZ, Dunstan DW, et al. Differences in height explain gender differences in the response to the oral glucose tolerance test- the AusDiab study. *Diabet Med* 2008;25(3):296-302.
22. Henderson-Smart DJ, Hutchinson JL, Donoghue DA, et al. Prenatal predictors of chronic lung disease in very preterm infants. *Arch Dis Child Fetal Neonatal Ed* 2006;91(1):F40-5.
23. Johnson S, Hollis C, Kochhar P, et al. Autism spectrum disorders in extremely preterm children. *J Pediatr* 2010;156(4):525-31 e2.
24. Mohamed MA, Aly H. Male gender is associated with intraventricular hemorrhage. *Pediatrics* 2010;125(2):e333-9.
25. Morse SB, Wu SS, Ma C, et al. Racial and gender differences in the viability of extremely low birth weight infants: a population-based study. *Pediatrics* 2006;117(1):e106-12.
26. Spinillo A, Montanari L, Gardella B, et al. Infant sex, obstetric risk factors, and 2-year neurodevelopmental outcome among preterm infants. *Dev Med Child Neurol* 2009;51(7):518-25.
27. Tyson JE, Parikh NA, Langer J, et al. Intensive care for extreme prematurity--moving beyond gestational age. *N Engl J Med* 2008;358(16):1672-81.

28. The Ministry of Education, Culture, Sports, Science, and Technology in Japan. A report of school health statistics, 2004.
29. Leger J, Levy-Marchal C, Bloch J, et al. Reduced final height and indications for insulin resistance in 20 year olds born small for gestational age: regional cohort study. *BMJ* 1997;315(7104):341-7.
30. Saenger P, Czernichow P, Hughes I, et al. Small for gestational age: short stature and beyond. *Endocr Rev* 2007;28(2):219-51.
31. The Statistics and Information Department, Minister's Secretariat, Ministry of Health, Labour, and Welfare. Vital Statistics in Japan, 2009. Vol.1. pp 124-127.
32. Crowther NJ, Cameron N, Trusler J, et al. Influence of catch-up growth on glucose tolerance and beta-cell function in 7-year-old children: results from the birth to twenty study. *Pediatrics* 2008;121(6):e1715-22.
33. Eriksson JG, Osmond C, Kajantie E, et al. Patterns of growth among children who later develop type 2 diabetes or its risk factors. *Diabetologia* 2006;49(12):2853-8.
34. Finken MJ, Keijzer-Veen MG, Dekker FW, et al. Preterm birth and later insulin resistance: effects of birth weight and postnatal growth in a population based longitudinal study from birth into adult life. *Diabetologia* 2006;49(3):478-85.
35. Leunissen RW, Kerkhof GF, Stijnen T, et al. Timing and tempo of first-year rapid growth in relation to cardiovascular and metabolic risk profile in early adulthood. *JAMA* 2009;301(21):2234-42.

Table 1. Clinical characteristics of young adults with very low birth weight (VLBW) at birth and at study assessment

	Total	Men (n=42)		Women (n=69)	
	(n=111)	SGA* (n=14)	AGA (n=28)	SGA* (n=25)	AGA (n=44)
At birth					
Gestational Age (wk)	29.7 (3.1)	31.5 (3.7)	28.1 (1.9)	33.0 (2.6)	28.2 (1.9)
Weight (g)	1152 (235)	1050 (223)	1149 (235)	1190 (239)	1166 (236)
ELBW	33 (29.7)	6 (42.9)	10 (35.7)	7 (28.0)	10 (22.7)
Birth from multiple pregnancy	18 (16.2)	2 (21.4)	7 (25.0)	1 (4.0)	8 (18.1)
At study assessment					
Age (yr)	24.8 (3.0)	24.9 (2.9)	24.6 (3.6)	23.9 (2.9)	25.4 (2.7)
Family history of diabetes	22 (19.8)	2 (14.3)	5 (17.9)	7 (28.0)	8 (18.2)
Height (m)	1.58 (0.07)	1.60 (0.06)	1.66 (0.06)	1.54 (0.06)	1.55 (0.05)
Body Weight (kg)	52.3 (10.1)	53.1 (8.8)	55.8 (8.9)	47.5 (8.3)	52.4 (11.3)
BMI (kg/m^2)	20.9 (3.8)	20.8 (3.2)	20.3 (2.8)	20.1 (3.0)	21.7 (4.7)
Blood pressure (mmHg)					
Systolic	118 (16)	120 (18)	121 (17)	115 (12)	117 (16)
Diastolic	70 (11)	69 (15)	69 (11)	69 (11)	71 (11)
Cholesterol (mg/dl)					
Total	184.8 (31.3)	210.6 (52.0)	183.4 (25.2)	180.4 (27.2)	179.9 (25.1)
LDL	104.3 (28.4)	126.9 (48.6)	104.8 (24.8)	100.3 (21.5)	99.0 (22.6)
HDL	65.7 (13.3)	63.2 (14.0)	64.6 (14.1)	64.6 (15.1)	67.8 (11.6)
Triglycerides (mg/dl)	81.8 (72.2)	126.5 (139.9)	81.0 (84.5)	85.4 (51.0)	65.9 (23.5)
Renal function					
Creatinine (mg/dl)	0.66 (0.13)	0.76 (0.09)	0.80 (0.11)	0.59 (0.08)	0.58 (0.08)
eGFR (ml/min/1.73 m^2)†	104.6 (18.4)	105.5 (13.9)	101.8 (19.4)	104.3 (18.2)	106.3 (19.5)

SGA, small for gestational age; AGA, appropriate for gestational age; ELBW, extremely low birth weight (<1000g); BMI, body mass index; eGFR, estimated glomerular filtration rate; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

Data are expressed as mean (SD) or number (%).

*Determined by a birth weight below the 10th percentile for gestational age according to standards defined by a study group of the Health Ministry in Japan.

†Calculated according to the formula recommended by the Japanese Society of Nephrology: eGFR (ml/min/1.73m^2) = 194 × Cre$^{-1.094}$ × Age$^{-0.287}$ (× 0.739 if the subject is a woman).

Table 2. Correlated factors for hyperglycemia* in young adults with very low birth weight (VLBW) assessed by logistic regression analyses

Variable	Odds Ratio†	95% CI	P-value
Gender (Male)	3.34	1.08 - 10.3	0.036
Factors at birth			
Gestational age (wk)	0.77	0.53 - 1.12	0.165
Weight (0.1kg)	1.39	0.96 - 2.02	0.085
SGA	2.56	0.37 - 17.5	0.340
Factors at study assessment			
Family history of diabetes	1.92	0.49 - 7.57	0.353
BMI (kg/m^2)	1.29	1.11 - 1.49	0.001

SGA, small for gestational age; BMI, body mass index.

*A category of hyperglycemia includes Diabetes, IGT (impaired glucose tolerance), IFG (impaired fasting glycaemia), and Non-diabetes/IGT/IFG with elevated 1-hour glucose levels (> 8.6mmol/l).

†Each odds ratio is calculated from a model including gender, family history of diabetes, BMI, gestational age, birth weight, and SGA/AGA (appropriate for gestational age).

Table 3. Gender differences in glucose regulation in young adults with very low birth weight (VLBW)

	Men (n=42)	Women (n=69)	P-value
Hyperglycemia	11 (26.2)	10 (14.5)	0.127
Diabetes	0	1	
IGT	2	4	
IFG	1	0	
Non-diabetes/IGT/IFG with elevated 1-hour glucose levels*	8	5	
HbA1c (%)	5.39 (5.31 - 5.47)	5.39 (5.31 - 5.47)	0.635
HOMA-β	72.5 (59.0 - 86.0)	103 (87.2 - 119.6)	0.001
HOMA-IR	1.4 (1.14 - 1.69)	1.6 (1.29 - 1.90)	0.477
Variable during OGTT			
Insulinogenic Index	1.1 (0.64 - 1.60)	1.4 (1.18 - 1.71)	0.002
$Glucose_{AUC}$ (mmol/l ×hr)	18.8 (17.9 - 19.7)	18.2 (17.3 - 19.0)	0.089
$Insulin_{AUC}$ (μU/ml × hr)	135.3 (98.7 - 171.9)	145.1 (121.7 - 168.6)	0.052
Reactive hypoglycemia	17 (40)	17 (25)	0.079

IGT, impaired glucose tolerance; IFG, impaired fasting glycemia; HOMA-β, homeostasis model of assessment for beta cell; HOMA-IR, homeostasis model of assessment for insulin resistance.

Data are expressed as mean (95% CI) or number (%).

*Defined as 1-hour glucose levels > 8.6mmol/l.

Figure Legends

Figure 1. Flow of participants through the study.

(There is no figure legend in Figure 1.)

Figure 2. The gender differences of glucose and insulin levels during OGTT in young adults with very low birth weight (VLBW).

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. 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).

The numerical data of Figure 2.

Male (n=42)

	0 (min)	30	60	90	120	180
Plasma glucose (mg/dl)	5.208 (0.3695)	8.579 (1.272)	7.167 (1.824)	6.262 (1.523)	5.749 (1.102)	4.360 (1.205)
Insulin (microU/ml)	6.057 (3.519)	64.95 (64.53)	53.70 (47.86)	53.27 (49.61)	49.84 (50.66)	20.94 (24.37)

Female (n=69)

	0 (min)	30	60	90	120	180
Plasma glucose (mg/dl)	4.930 (0.4266)	7.529 (1.460)	6.596 (1.946)	6.178 (1.541)	5.829 (1.487)	4.881 (1.121)
Insulin (microU/ml)	7.090 (5.107)	66.22 (42.62)	57.09 (51.97)	54.95 (44.39)	51.11 (39.85)	31.82 (28.03)

Data are expressed as mean (SD).

Figure 1. Flow of Participants through the study.

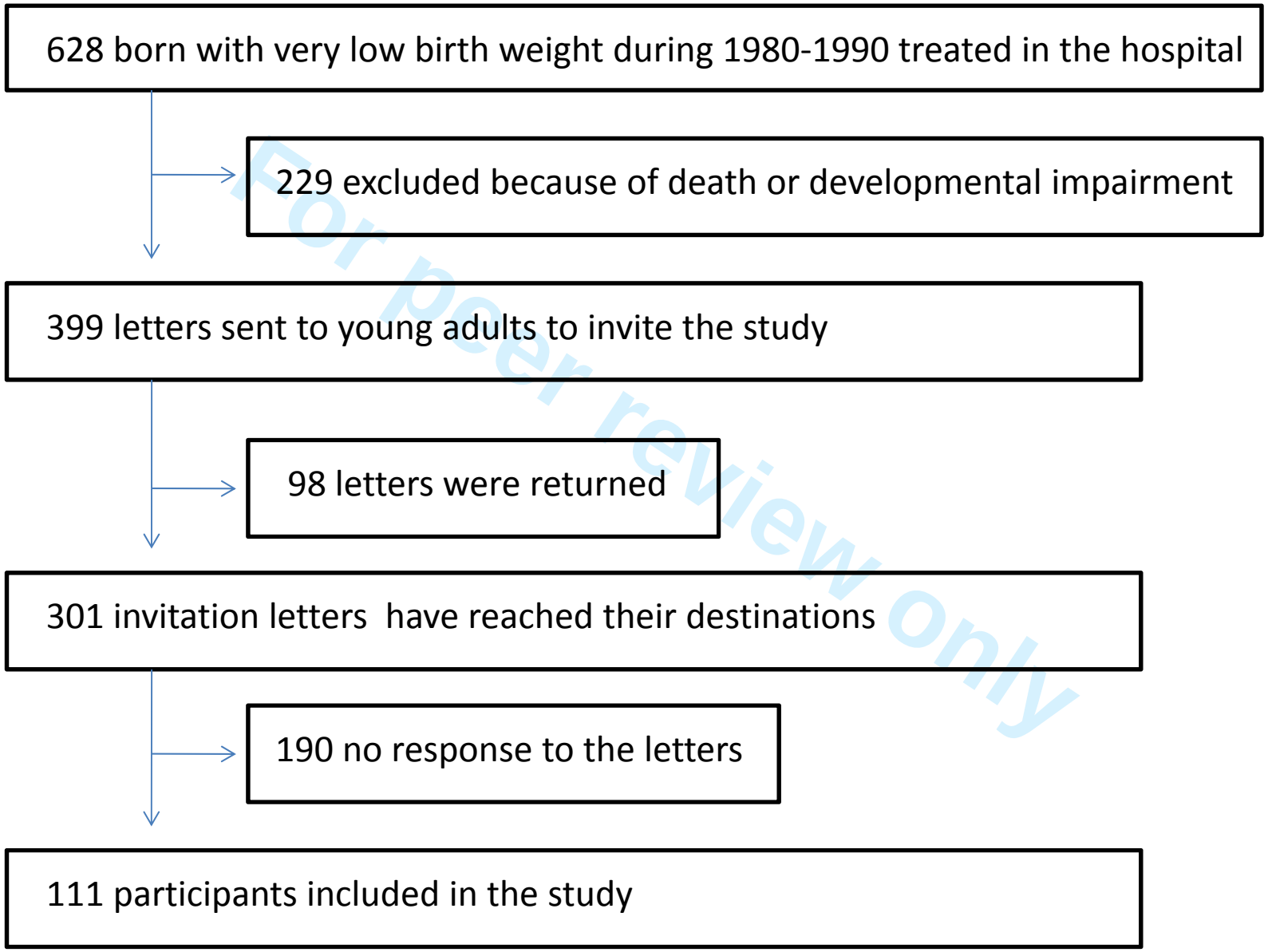
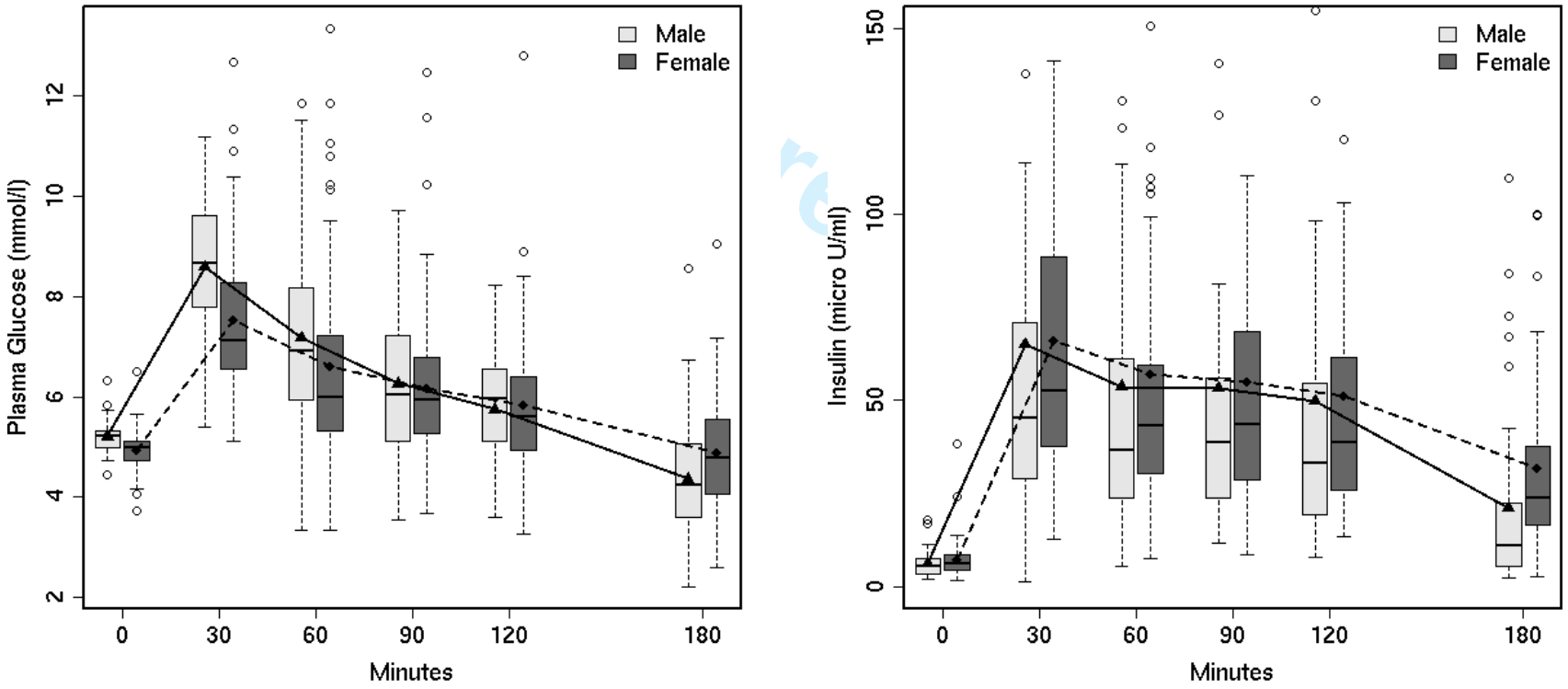


Figure 2. The gender differences of glucose and insulin levels during OGTT in young adults with very low birth weight (VLBW).



STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	7
Objectives	3	State specific objectives, including any prespecified hypotheses	7
Methods			
Study design	4	Present key elements of study design early in the paper	8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	8
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	8
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	9-10
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	n/a
Bias	9	Describe any efforts to address potential sources of bias	18
Study size	10	Explain how the study size was arrived at	8
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	10
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	10-12
		(*b*) Describe any methods used to examine subgroups and interactions	13
		(*c*) Explain how missing data were addressed	n/a
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	n/a
		(*e*) Describe any sensitivity analyses	n/a
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	8
		(c) Consider use of a flow diagram	Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1
		(b) Indicate number of participants with missing data for each variable of interest	n/a
Outcome data	15*	Report numbers of outcome events or summary measures	n/a
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	12-14
		(*b*) Report category boundaries when continuous variables were categorized	n/a
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	14
Discussion			
Key results	18	Summarise key results with reference to study objectives	14-15
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	17-18
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14-18
Generalisability	21	Discuss the generalisability (external validity) of the study results	17
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	19

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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

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

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ABSTRACT

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

Design: Cross-sectional, observational study.

Setting: A general hospital in Hamamatsu, Japan.

Participants: 111 young adults (42 men and 69 women; aged 19-30 years) born with VLBW between 1980 and 1990. Participants underwent standard 75-g oral glucose tolerance test (OGTT).

Primary and secondary outcome measures: The primary outcomes were glucose and insulin levels during OGTT, and risk factors for a category of hyperglycemia defined as follows: diabetes mellitus; impaired glucose tolerance (IGT); impaired fasting glycemia (IFG); and non-diabetes/IGT/IFG with elevated 1-hour glucose levels (>8.6 mmol/l). The secondary outcomes were the pancreatic beta cell function (insulinogenic index and homeostasis model of assessment for beta cell [HOMA-β]), and insulin resistance (homeostasis model of assessment for insulin resistance [HOMA-IR]).

Results: Of 111 young adults with VLBW, 21 subjects (19%) had hyperglycemia: 1 had type 2 diabetes; 6 had IGT; 1 had IFG; and 13 had non-diabetes/IGT/IFG with elevated 1-hour glucose levels. In logistic regression analysis, male gender was an independent

risk factor associated with hyperglycemia (OR: 3.34; 95%CI: 1.08-10.3; p=0.036). 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 measures ANOVA). Pancreatic beta cell function was lower in men (insulinogenic index: p=0.002; HOMA-β: p=0.001), although no gender difference was found in insulin resistance (HOMA-IR: p=0.477). In male subjects, logistic regression analysis showed that small for gestational age (SGA) was an independent risk factor associated with hyperglycemia (OR: 33.3; 95%CI: 1.67-662.6; p=0.022).

Conclusions: 19% of individuals with VLBW already had hyperglycemia in young adulthood, and male gender was a significant independent risk factor of hyperglycemia. In male young adults with VLBW, SGA was associated with hyperglycemia.

ARTICLE SUMMARY

Article focus

- Neonatal intensive care has improved the survival rate for very low birth weight infants (VLBW; birth weight <1500g) in recent decades, and the first generation of VLBW infants have only recently reached young adulthood.
- 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.
- The present study investigated glucose regulation in young adults with VLBW in an Asian population, and determined the factors associated with hyperglycemia.

Key messages

- Of 111 young adults with VLBW, 19% of individuals already had hyperglycemia (type 2 diabetes, IGT, IFG, and non-diabetes/IGT/IFG with elevated 1-hour glucose levels).
- Male gender was a significant independent risk factor of hyperglycemia in young adults with VLBW.
- Small for gestational age (SGA) was associated with hyperglycemia particularly in

male young adults with VLBW.

Strengths and limitations of this study

- This is the first study assessing the glucose regulation in young adults with VLBW in an Asian population.
- This study does not provide information on postnatal growth patterns, which have been shown to be associated with later hyperglycemia in previous studies.
- The study design with no control subjects makes it impossible to address the delayed impact of VLBW itself on glucose regulation.

INTRODUCTION

In recent decades, progression of neonatal intensive care has dramatically increased the survival rate of very low birth weight (VLBW; birth weight <1500g) infants worldwide.[1] A lot of them have grown up into young adult (now in their twenties or thirties). To date, epidemiological studies have shown an association between low birth weight and type 2 diabetes and cardiovascular disease in later life.[2-4] Fetal malnutrition in the gestational period, which prevents appropriate fetal growth in utero, is thought to provoke thrifty phenotype in premature babies. This phenotype is assumed to predispose them to subsequent metabolic disorders. 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.[5-7]

The first generation of VLBW infants have only recently reached young adulthood. A few studies have shown that VLBW (or preterm) is associated with glucose intolerance in young adulthood in Caucasian populations,[8 9] while the glucose regulation in Asian young adults with VLBW remains uncertain. To clarify the characteristics of glucose metabolism in young adults with VLBW in an Asian population, we investigated glucose regulation in 111 young adults with VLBW by performing detailed oral glucose tolerance test (OGTT), which is useful for evaluation of early signs of glucose

intolerance.

METHODS

Study Participants

The birth record database of Seirei Hamamatsu General Hospital (Hamamatsu, Japan) showed that 628 subjects were born with VLBW between 1980 and 1990, and were treated at a neonatal intensive care unit (**Figure 1**). VLBW infants were defined according to the World Health Organization (WHO) criteria: babies whose birth weight was less than 1500g. Out of the 628 subjects, 229 were excluded because of death (n=132) or severe neurodevelopmental impairment (n=97). To the remaining 399 subjects, we sent letters which provided information regarding the study and requested their participation. Among the 399 letters, 98 were returned marked as address unknown (i.e. the remaining 301 letters were thought to reach their destinations). Consequently, 111 subjects (aged 19-30 years) participated in the present study. All participants were Japanese. Small for gestational age (SGA) status was determined according to standards by a study group of the Health Ministry in Japan: a birth weight below the 10th percentile for gestational age.[10] The basal characteristics of participants at birth are summarized in **Table 1**.

Measurements

All participants underwent a standard 75-g OGTT after a ten-hour overnight fast. Plasma glucose and serum insulin concentrations were examined at 0, 30, 60, 90, 120, and 180 minutes during OGTT. Fasting glucose levels and 2-hour glucose levels were used for diagnosing diabetes mellitus, impaired glucose tolerance (IGT), and impaired fasting glycaemia (IFG) according to the WHO criteria.[11] Since it has been shown that 1-hour plasma glucose concentration is associated with future risk of type 2 diabetes and atherosclerosis, 1-hour plasma glucose above 8.6 mmol/l (155 mg/dl) was included as a category of hyperglycemia.[12 13] Reactive hypoglycemia during OGTT was defined as the level of plasma glucose less than 3.8 mmol/l which causes the response of counter-regulatory hormone release.[14]

We measured plasma glucose and serum insulin levels during OGTT with an autoanalyzer JCA-BM2250 (JEOL, Tokyo, Japan). Plasma glucose was measured by means of hexokinase method. The concentration of serum insulin was measured with chemiluminescent enzyme immunoassay (CLIA). Fasting blood samples were also drawn for other measurements; total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglyceride, and creatinine.

Glycated haemoglobin A1c (HbA1c) was measured with high-performance liquid chromatography (HPLC) method using an automated glycohaemoglobin analyzer HLC-723G8 (Tosoh Bioscience, Tokyo, Japan). The values for HbA1c were converted from Japanese Diabetes Society (JDS) values into National Glycohaemoglobin Standardization Program (NGSP) equivalent values. NGSP equivalent values were calculated with the formula: HbA1c (%) = JDS value (%) +0.4.[15]

Calculations and Statistical analysis

Pancreatic beta cell function was evaluated by both insulinogenic index and homeostasis model of assessment for beta cell (HOMA-β).[16] Insulinogenic index, the index of early-phase insulin secretion, was calculated as the ratio of the increment in insulin concentration to the increment in glucose concentration ([30-min insulin (μU/ml)–fasting insulin]/[30-min glucose (mmol/l) – fasting glucose]). HOMA-β was calculated as follows: 20 × fasting insulin (μU/ml) / [fasting glucose (mmol/l) –3.5]. Insulin resistance was estimated by homeostasis model of assessment for insulin resistance (HOMA-IR): fasting insulin (μU/ml) × fasting glucose (mmol/l)/22.5.[16] The total amounts of glucose and insulin levels during OGTT were assessed by calculating areas under the curve (AUC) with trapezoid rules. The estimated glomerular filtration

rate (eGFR) was calculated according to the following formula, as recommended by the Japanese Society of Nephrology: eGFR (ml/min/1.73m^2) = 194 × [Cre (mg/dl)]$^{-1.094}$ × [Age (years)]$^{-0.287}$ (× 0.739 if the subject is a woman).[17]

Quantitative variables were expressed as mean and standard deviation (SD) or 95% confidence interval (CI); categorical variables were presented as number and percentage. Differences between groups were compared using the Student's t-test, the Mann–Whitney U test, the Pearson's χ^2 test, or the repeated measures analysis of variance (ANOVA) as appropriate. The data on insulin levels during OGTT were logarithmically transformed before the repeated measures ANOVA. Logistic regression analysis which included gender, family history of diabetes within the second degree, BMI, gestational age, birth weight, and SGA/AGA (appropriate for gestational age) was performed to estimate odds ratios for the category of hyperglycemia. For the further investigation into glucose regulation, multiple linear regression analysis was conducted. Gender, family history of diabetes, BMI, gestational age, birth weight, and SGA/AGA were included in the model. The data on HOMA-β, HOMA-IR, Insulinogenic Index, and $Glucose_{AUC}$ were logarithmically transformed before analysis to meet the assumptions of normality. A p-value of less than 0.05 was defined as statistically significant. All analyses were conducted using SAS software version 9.2 (SAS Institute,

Cary, North Carolina, USA) and the statistical software R version 2.12.2 (http://www.r-project.org).

RESULTS

The basic characteristics of participants at study assessment are shown in **Table 1**. Of 111 young adults with VLBW, 21 subjects (19%) had hyperglycemia: one had type 2 diabetes; six had impaired glucose tolerance (IGT); one had impaired fasting glycaemia (IFG); 13 non-diabetes/IGT/IFG subjects had elevated 1-hour glucose levels (>8.6 mmol/l). Hyperglycemia was more frequent in men than women (26.2 % for men vs 14.5 % for women). In the logistic regression analysis adjusted for family history of diabetes within the second degree, BMI, gestational age, birth weight and SGA/AGA, male gender was a statistically significant independent factor associated with hyperglycemia (Odds ratio: 3.34; 95% CI: 1.08-10.3; p= 0.036). BMI at study assessment was also associated with hyperglycemia (**Table 2**).

Gender difference

As male gender was a significant independent risk factor of hyperglycemia, we evaluated the differences in glucose regulation between men and women in the sample

group. **Figure 2** shows the glucose and insulin response during OGTT in men and women. Male subjects had significantly higher levels of glucose during OGTT than female subjects ($p<0.001$ by repeated measures ANOVA). In terms of insulin levels, male subjects had lower levels of insulin during OGTT than female subjects ($p=0.005$ by repeated measures ANOVA). $Glucose_{AUC}$ during OGTT tended to be higher in male subjects (**Table 3**). As for the function of insulin secretion, insulinogenic index and HOMA-ß were significantly lower in men than in women. $Insulin_{AUC}$ also showed a tendency to be lower in male subjects. Reactive hypoglycemia during OGTT tended to be frequent in men. The differences in the mean values of HbA1c and HOMA-IR were not statistically significant. There were no significant gender differences in gestational age ($p=0.145$), birth weight ($p=0.168$), age at study assessment ($p=0.845$), BMI ($p=0.879$), the proportion of SGA ($p=0.756$), and that of family history of diabetes within the second degree ($p=0.516$). The variables for glucose metabolism in men and women are summarized in **Table 3.**

We evaluated the associations between gender and the variables of glucose metabolism by multiple linear regression analysis. Adjustments were made for family history of diabetes within the second degree, BMI, gestational age, birth weight, and SGA/AGA. In this analysis, male gender had inverse associations with HOMA-β (β: -0.336; 95%

CI: -0.509 to -0.163; p< 0.001) and insulinogenic index (β: -0.195; 95% CI: -0.344 to -0.047; p=0.01). $Glucose_{AUC}$ during OGTT tended to be positively associated with male gender (β: 0.056; 95% CI: -0.0047 to 0.116; p=0.071).

In male subjects, after an adjustment for family history of diabetes, BMI, gestational age, and birth weight, the logistic regression analysis showed that SGA was a statistically significant independent factor associated with hyperglycemia (Odds ratio: 33.3; 95% CI: 1.67-662.6; p=0.022).

DISCUSSION

To the best of our knowledge, this is the first study assessing the glucose regulation in young adults with VLBW in an Asian population. Our study has indicated that 19% of young adults with VLBW already had hyperglycemia: type 2 diabetes; IGT; IFG; and non-diabetes/IGT/IFG with high 1-hour plasma glucose level. A report from the Japanese Ministry of Health, Welfare, and Labour in 2007 showed that of 204 general young adults (aged 20-29 years), 2 individuals (0.98%) had high levels of HbA1c (more than 6.0%; NGSP equivalent values),[18] while 3.6% of young adults with VLBW had the HbA1c values more than 6.0% in the present study. In a previous study, Hovi et al. reported that VLBW infants in young adulthood had higher indexes of glucose

intolerance compared with term infants.[9] A recent epidemiological study has also shown that preterm birth is associated with an increased risk of diabetes in young adults.[8] On the other hand, a study in the Netherlands showed that preterm birth was not associated with reduced insulin sensitivity in young adulthood.[19] The findings of that study may be biased by the way of recruiting the control subjects born at term. Our findings would be in line with the standpoint of high prevalence of hyperglycemia in premature infants in young adulthood, although absence of control subjects presents a limitation of demonstrating the impact of VLBW itself on glucose regulation.

We have also found that male subjects had higher glucose levels during OGTT than females. Previous studies in the general population showed that women had higher postload glucose levels than men, which were explained by differences in body size.[20 21] During standard 75-g OGTT, men and women take the same amount of glucose, which is thought to be high dosage for women relative to their body size. In our study, however, men had significantly higher levels of both fasting and postload glucose concentrations during OGTT. Moreover, male gender was associated with lower beta cell function and the risk of glucose intolerance. These findings might indicate that men with VLBW are more predisposed to diabetes than women; indeed, recent studies have shown that male premature infants are more vulnerable than females.[22-27] In particular,

the male sex is associated with various adverse outcomes including death,[25 27] respiratory dysfunction,[22] intraventricular hemorrhage,[24] autism spectrum,[23] and neurodevelopmental impairment.[26] Interestingly, in the present study, the mean value of height (155cm) in women with VLBW is close to the average value of the Japanese female population (158cm), whereas men with VLBW (164cm) were found to be shorter compared to the Japanese male population (171cm) (average height data of Japanese population were drawn from the report by the Ministry of Education, Culture, Sports, Science, and Technology in Japan).[28] In a previous study, young adults who had been born SGA were shorter and had higher glucose levels than those with a normal birth weight.[29] Reduced final height might be long term consequences of intrauterine retardation, which would also influence glucose regulation. Further investigation is needed to clarify whether the influence of VLBW on physical growth is more remarkable in male than female infants, and elucidate the relationship between physical growth and glucose regulation.

Previous studies have shown that SGA itself is associated with glucose intolerance.[30] In our study, SGA was not significantly associated with hyperglycemia in total subjects, but was associated in male subjects. The influence of SGA on glucose regulation in young adults with VLBW might be stronger in male than females. It is possible that this

gender difference in glucose regulation is owing to the gender difference in the strength of SGA effect.

Strength and weakness of the study

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).[31] Our study includes a relatively large number of young adults with VLBW, and the individuals with profound complications were carefully excluded in the recruiting process. In addition, as participants in the cohort were all Japanese, their racial homogeneity made considerations of ethnic differences in glucose regulation unnecessary. To date, the glucose regulation of Asian young adults with VLBW has been uncertain. Our findings would be useful to clinicians and researchers, and stimulate future large-scale prospective cohort studies in Asian populations.

In the present study, we could not obtain information regarding the growth rate in childhood of all participants. This is a major limitation of the study. The postnatal growth pattern in infancy has been shown to be associated with later glucose intolerance.[32-35] The clinical records of participants were written 20 to 30 years ago, and some of them were no longer preserved. Additionally, maternal factors such as advanced

age, smoking, gestational diabetes, and perinatal complications were not available in the present study. These factors might have affected fetal malnutrition and subsequently led to VLBW. 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. Another concern of the study is selection bias. Of 301 VLBW subjects who were thought to receive the invitation letters, 111 subjects (37%) 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.

Future Perspective

As neonatal intensive care is making steady progress, an increasing number of young adults with VLBW worldwide will face a greater variety of health problems. Clinician should be aware of the risk of hyperglycemia in young adults with VLBW, and follow up them for a longer period of time. It may be worthwhile for them to check their glucose metabolism with OGTT in their twenties or thirties, which would lead to early intervention in their lifestyle and subsequently contribute to prevention of type 2 diabetes and cardiovascular disease.[5-7] In the present study, we have found that male

gender was a significant independent risk factor of hyperglycemia in young adults with VLBW. In addition to the gender difference, future studies are required to focus on the factors affecting the glucose metabolism in VLBW infants.

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Contributors: RS and HW drafted the paper. RS conceived the study. RS and KS developed the idea. RS, HW, KS, SO, RG, MM, and HN were involved in study design. RS and KS participated in data acquisition. RS, HW, HM, EI, MT, and MM were engaged in data analysis. All the authors contributed to the interpretation of the findings. All the authors were involved in the revision and approved the final version.

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Promotion of Science (21390180) and from the Ministry of Health, Labour and Welfare in Japan (H23-Cardiovascular disease and Diabetes-011).

Competing Interest: None.

Ethical approval: The study was approved by the ethical committee of Seirei Hamamatsu General Hospital. All participants provided written informed consent to participate in the study.

Data sharing statement: Data will not be publically accessible. However, interested individuals may contact the authors.

REFERENCES

1. Field DJ, Dorling JS, Manktelow BN, et al. Survival of extremely premature babies in a geographically defined population: prospective cohort study of 1994-9 compared with 2000-5. *BMJ* 2008;336(7655):1221-3.
2. Barker DJ, Gluckman PD, Godfrey KM,et al. Fetal nutrition and cardiovascular disease in adult life. *Lancet* 1993;341(8850):938-41.
3. Kaijser M, Bonamy AK, Akre O, et al. Perinatal risk factors for diabetes in later life. *Diabetes* 2009;58(3):523-6.
4. Whincup PH, Kaye SJ, Owen CG, et al. Birth weight and risk of type 2 diabetes: a systematic review. *JAMA* 2008;300(24):2886-97.
5. Knowler WC, Barrett-Connor E, Fowler SE, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* 2002;346(6):393-403.
6. Eriksson KF, Lindgarde F. Prevention of type 2 (non-insulin-dependent) diabetes mellitus by diet and physical exercise. The 6-year Malmo feasibility study. *Diabetologia* 1991;34(12):891-8.
7. Tuomilehto J, Lindstrom J, Eriksson JG, et al. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N Engl J Med* 2001;344(18):1343-50.
8. Crump C, Winkleby MA, Sundquist K, et al. Risk of diabetes among young adults born preterm in Sweden. *Diabetes Care* 2011;34(5):1109-13.
9. Hovi P, Andersson S, Eriksson JG, et al. Glucose regulation in young adults with very low birth weight. *N Engl J Med* 2007;356(20):2053-63.
10. The study group of the Health Ministry in Japan. Fetal growth curves of Japanese, 1994. pp 61.
11. World Health Organization. Definition, diagnosis and classification of diabetes mellitus and its complications. Geneva: World Health Organization, 1999.
12. Abdul-Ghani MA, Abdul-Ghani T, Ali N, et al. One-hour plasma glucose concentration and the metabolic syndrome identify subjects at high risk for future type 2 diabetes. *Diabetes Care* 2008;31(8):1650-5.
13. Succurro E, Marini MA, Arturi F, et al. Elevated one-hour post-load plasma glucose levels identifies subjects with normal glucose tolerance but early carotid atherosclerosis. *Atherosclerosis* 2009;207(1):245-9.
14. Zammitt NN, Frier BM. Hypoglycemia in type 2 diabetes: pathophysiology, frequency, and effects of different treatment modalities. *Diabetes Care*

2005;28(12):2948-61.

15. The Committee of Japan Diabetes Society on the diagnostic criteria of diabetes mellitus. Report of the Committee on the classification and diagnostic criteria of diabetes mellitus. *J Japan Diab Soc* 2010; 53 450-67.
16. Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28(7):412-9.
17. Matsuo S, Imai E, Horio M, et al. Revised equations for estimated GFR from serum creatinine in Japan. *Am J Kidney Dis* 2009;53(6):982-92.
18. The Ministry of Health, Labour, and Welfare. The National Health and Nutrition Survey in Japan, 2007. pp 187.
19. Willemsen RH, Leunissen RW, Stijnen T, et al. Prematurity is not associated with reduced insulin sensitivity in adulthood. *J Clin Endocrinol Metab* 2009;94(5):1695-700.
20. Faerch K, Borch-Johnsen K, Vaag A, et al. Sex differences in glucose levels: a consequence of physiology or methodological convenience? The Inter99 study. *Diabetologia* 2010;53(5):858-65.
21. Sicree RA, Zimmet PZ, Dunstan DW, et al. Differences in height explain gender differences in the response to the oral glucose tolerance test- the AusDiab study. *Diabet Med* 2008;25(3):296-302.
22. Henderson-Smart DJ, Hutchinson JL, Donoghue DA, et al. Prenatal predictors of chronic lung disease in very preterm infants. *Arch Dis Child Fetal Neonatal Ed* 2006;91(1):F40-5.
23. Johnson S, Hollis C, Kochhar P, et al. Autism spectrum disorders in extremely preterm children. *J Pediatr* 2010;156(4):525-31 e2.
24. Mohamed MA, Aly H. Male gender is associated with intraventricular hemorrhage. *Pediatrics* 2010;125(2):e333-9.
25. Morse SB, Wu SS, Ma C, et al. Racial and gender differences in the viability of extremely low birth weight infants: a population-based study. *Pediatrics* 2006;117(1):e106-12.
26. Spinillo A, Montanari L, Gardella B, et al. Infant sex, obstetric risk factors, and 2-year neurodevelopmental outcome among preterm infants. *Dev Med Child Neurol* 2009;51(7):518-25.
27. Tyson JE, Parikh NA, Langer J, et al. Intensive care for extreme prematurity--moving beyond gestational age. *N Engl J Med* 2008;358(16):1672-81.

28. The Ministry of Education, Culture, Sports, Science, and Technology in Japan. A report of school health statistics, 2004.
29. Leger J, Levy-Marchal C, Bloch J, et al. Reduced final height and indications for insulin resistance in 20 year olds born small for gestational age: regional cohort study. *BMJ* 1997;315(7104):341-7.
30. Saenger P, Czernichow P, Hughes I, et al. Small for gestational age: short stature and beyond. *Endocr Rev* 2007;28(2):219-51.
31. The Statistics and Information Department, Minister's Secretariat, Ministry of Health, Labour, and Welfare. Vital Statistics in Japan, 2009. Vol.1. pp 124-127.
32. Crowther NJ, Cameron N, Trusler J, et al. Influence of catch-up growth on glucose tolerance and beta-cell function in 7-year-old children: results from the birth to twenty study. *Pediatrics* 2008;121(6):e1715-22.
33. Eriksson JG, Osmond C, Kajantie E, et al. Patterns of growth among children who later develop type 2 diabetes or its risk factors. *Diabetologia* 2006;49(12):2853-8.
34. Finken MJ, Keijzer-Veen MG, Dekker FW, et al. Preterm birth and later insulin resistance: effects of birth weight and postnatal growth in a population based longitudinal study from birth into adult life. *Diabetologia* 2006;49(3):478-85.
35. Leunissen RW, Kerkhof GF, Stijnen T, et al. Timing and tempo of first-year rapid growth in relation to cardiovascular and metabolic risk profile in early adulthood. *JAMA* 2009;301(21):2234-42.

Table 1. Clinical characteristics of young adults with very low birth weight (VLBW) at birth and at study assessment

	Total	Men (n=42)		Women (n=69)	
	(n=111)	SGA* (n=14)	AGA (n=28)	SGA* (n=25)	AGA (n=44)
At birth					
Gestational Age (wk)	29.7 (3.1)	31.5 (3.7)	28.1 (1.9)	33.0 (2.6)	28.2 (1.9)
Weight (g)	1152 (235)	1050 (223)	1149 (235)	1190 (239)	1166 (236)
ELBW	33 (29.7)	6 (42.9)	10 (35.7)	7 (28.0)	10 (22.7)
Birth from multiple pregnancy	18 (16.2)	2 (21.4)	7 (25.0)	1 (4.0)	8 (18.1)
At study assessment					
Age (yr)	24.8 (3.0)	24.9 (2.9)	24.6 (3.6)	23.9 (2.9)	25.4 (2.7)
Family history of diabetes	22 (19.8)	2 (14.3)	5 (17.9)	7 (28.0)	8 (18.2)
Height (m)	1.58 (0.07)	1.60 (0.06)	1.66 (0.06)	1.54 (0.06)	1.55 (0.05)
Body Weight (kg)	52.3 (10.1)	53.1 (8.8)	55.8 (8.9)	47.5 (8.3)	52.4 (11.3)
BMI (kg/m^2)	20.9 (3.8)	20.8 (3.2)	20.3 (2.8)	20.1 (3.0)	21.7 (4.7)
Blood pressure (mmHg)					
Systolic	118 (16)	120 (18)	121 (17)	115 (12)	117 (16)
Diastolic	70 (11)	69 (15)	69 (11)	69 (11)	71 (11)
Cholesterol (mg/dl)					
Total	184.8 (31.3)	210.6 (52.0)	183.4 (25.2)	180.4 (27.2)	179.9 (25.1)
LDL	104.3 (28.4)	126.9 (48.6)	104.8 (24.8)	100.3 (21.5)	99.0 (22.6)
HDL	65.7 (13.3)	63.2 (14.0)	64.6 (14.1)	64.6 (15.1)	67.8 (11.6)
Triglycerides (mg/dl)	81.8 (72.2)	126.5 (139.9)	81.0 (84.5)	85.4 (51.0)	65.9 (23.5)
Renal function					
Creatinine (mg/dl)	0.66 (0.13)	0.76 (0.09)	0.80 (0.11)	0.59 (0.08)	0.58 (0.08)
eGFR (ml/min/1.73 m^2)†	104.6 (18.4)	105.5 (13.9)	101.8 (19.4)	104.3 (18.2)	106.3 (19.5)

SGA, small for gestational age; AGA, appropriate for gestational age; ELBW, extremely low birth weight (<1000g); BMI, body mass index; eGFR, estimated glomerular filtration rate; LDL, low-density lipoprotein; HDL, high-density lipoprotein.
Data are expressed as mean (SD) or number (%).

*Determined by a birth weight below the 10th percentile for gestational age according to standards defined by a study group of the Health Ministry in Japan.

†Calculated according to the formula recommended by the Japanese Society of Nephrology: eGFR (ml/min/1.73m^2) = 194 × [Cre (mg/dl)]$^{-1.094}$ × [Age (years)]$^{-0.287}$ (× 0.739 if the subject is a woman).

Table 2. Correlated factors for hyperglycemia* in young adults with very low birth weight (VLBW) assessed by logistic regression analyses

Variable	Odds Ratio†	95% CI	P-value
Gender (Male)	3.34	1.08 - 10.3	0.036
Factors at birth			
Gestational age (wk)	0.77	0.53 - 1.12	0.165
Weight (0.1kg)	1.39	0.96 - 2.02	0.085
SGA	2.56	0.37 - 17.5	0.340
Factors at study assessment			
Family history of diabetes	1.92	0.49 - 7.57	0.353
BMI (kg/m^2)	1.29	1.11 - 1.49	0.001

SGA, small for gestational age; BMI, body mass index.

*A category of hyperglycemia includes Diabetes, IGT (impaired glucose tolerance), IFG (impaired fasting glycaemia), and Non-diabetes/IGT/IFG with elevated 1-hour glucose levels (> 8.6mmol/l).

†Each odds ratio is calculated from a model including gender, family history of diabetes, BMI, gestational age, birth weight, and SGA/AGA (appropriate for gestational age).

Table 3. Gender differences in glucose regulation in young adults with very low birth weight (VLBW)

	Men (n=42)	Women (n=69)	P-value
Hyperglycemia	11 (26.2)	10 (14.5)	0.127
Diabetes	0	1	
IGT	2	4	
IFG	1	0	
Non-diabetes/IGT/IFG with elevated 1-hour glucose levels*	8	5	
HbA1c (%)	5.39 (5.31 - 5.47)	5.39 (5.31 - 5.47)	0.635
HOMA-β	72.5 (59.0 - 86.0)	103 (87.2 - 119.6)	0.001
HOMA-IR	1.4 (1.14 - 1.69)	1.6 (1.29 - 1.90)	0.477
Variable during OGTT			
Insulinogenic Index	1.1 (0.64 - 1.60)	1.4 (1.18 - 1.71)	0.002
$Glucose_{AUC}$ (mmol/l ×hr)	18.8 (17.9 - 19.7)	18.2 (17.3 - 19.0)	0.089
$Insulin_{AUC}$ (μU/ml × hr)	135.3 (98.7 - 171.9)	145.1 (121.7 - 168.6)	0.052
Reactive hypoglycemia	17 (40)	17 (25)	0.079

IGT, impaired glucose tolerance; IFG, impaired fasting glycemia; HOMA-β, homeostasis model of assessment for beta cell; HOMA-IR, homeostasis model of assessment for insulin resistance.

Data are expressed as mean (95% CI) or number (%).

*Defined as 1-hour glucose levels > 8.6mmol/l.

Figure Legends

Figure 1. Flow of participants through the study.

(There is no figure legend in Figure 1.)

Figure 2. The gender differences of glucose and insulin levels during OGTT in young adults with very low birth weight (VLBW).

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. 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).

The numerical data of Figure 2.

Male (n=42)

	0 (min)	30	60	90	120	180
Plasma glucose (mg/dl)	5.208 (0.3695)	8.579 (1.272)	7.167 (1.824)	6.262 (1.523)	5.749 (1.102)	4.360 (1.205)
Insulin (microU/ml)	6.057 (3.519)	64.95 (64.53)	53.70 (47.86)	53.27 (49.61)	49.84 (50.66)	20.94 (24.37)

Female (n=69)

	0 (min)	30	60	90	120	180
Plasma glucose (mg/dl)	4.930 (0.4266)	7.529 (1.460)	6.596 (1.946)	6.178 (1.541)	5.829 (1.487)	4.881 (1.121)
Insulin (microU/ml)	7.090 (5.107)	66.22 (42.62)	57.09 (51.97)	54.95 (44.39)	51.11 (39.85)	31.82 (28.03)

Data are expressed as mean (SD).

Figure 1. Flow of Participants through the study.

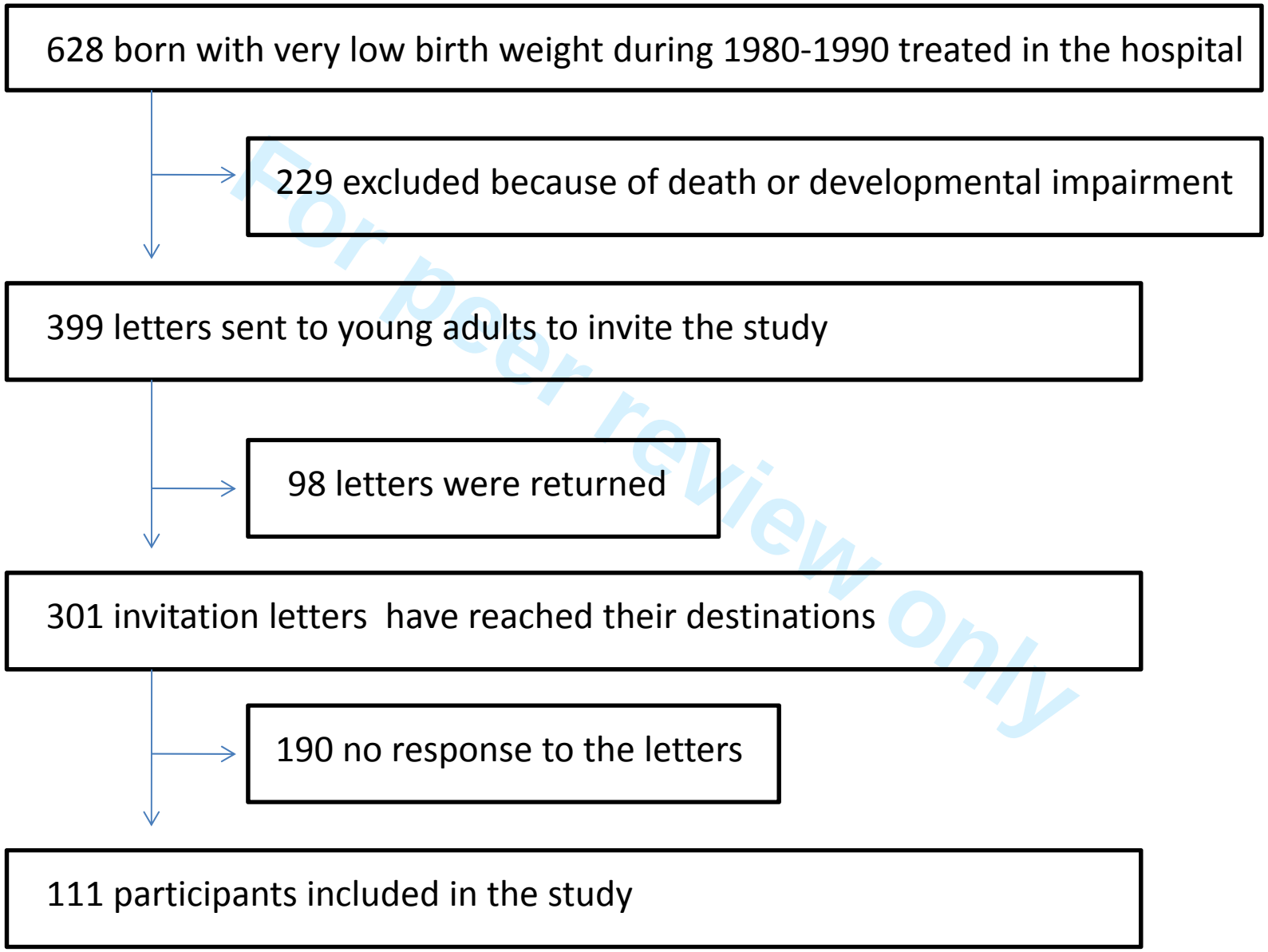
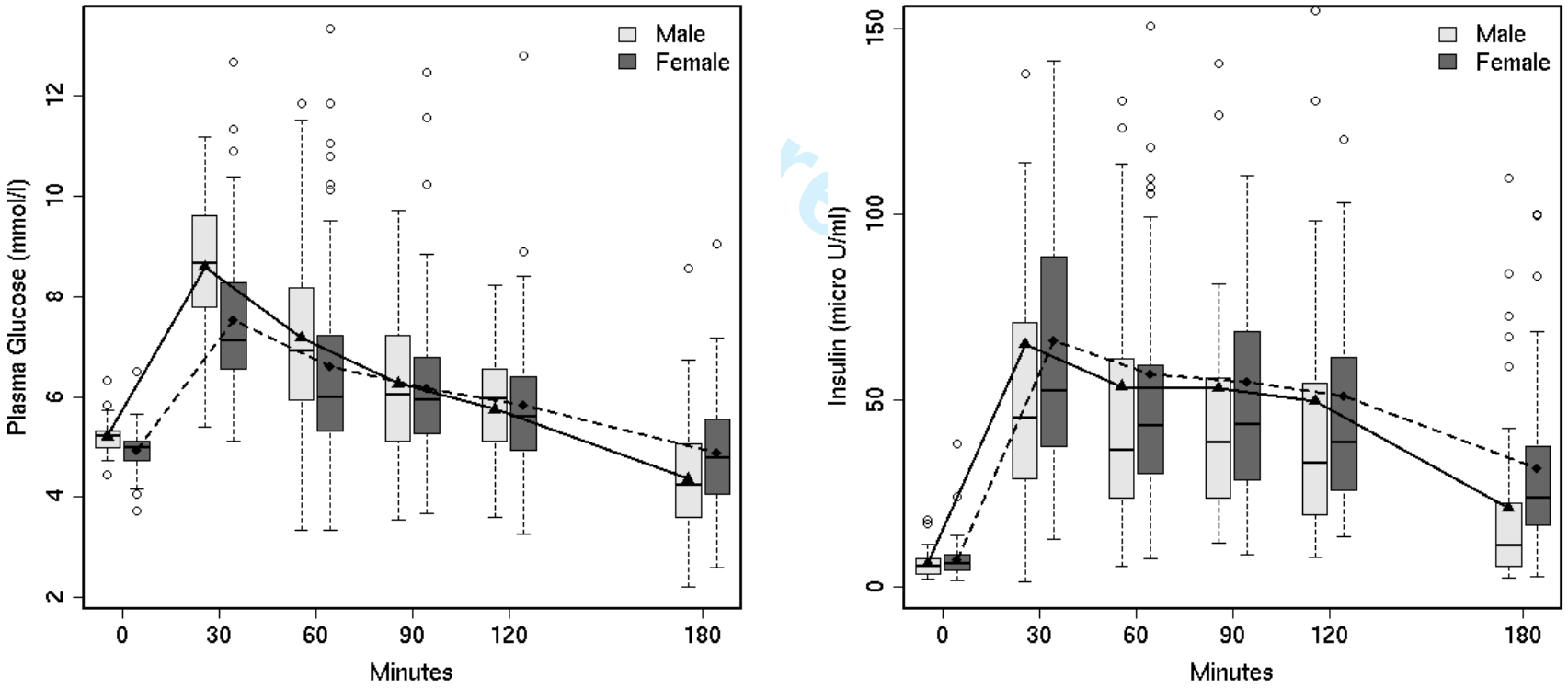


Figure 2. The gender differences of glucose and insulin levels during OGTT in young adults with very low birth weight (VLBW).



STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	7
Objectives	3	State specific objectives, including any prespecified hypotheses	7
Methods			
Study design	4	Present key elements of study design early in the paper	8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	8
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	8
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	9-10
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	n/a
Bias	9	Describe any efforts to address potential sources of bias	18
Study size	10	Explain how the study size was arrived at	8
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	10
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	10-12
		(*b*) Describe any methods used to examine subgroups and interactions	13
		(*c*) Explain how missing data were addressed	n/a
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	n/a
		(*e*) Describe any sensitivity analyses	n/a
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	8
		(c) Consider use of a flow diagram	Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1
		(b) Indicate number of participants with missing data for each variable of interest	n/a
Outcome data	15*	Report numbers of outcome events or summary measures	n/a
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	12-14
		(*b*) Report category boundaries when continuous variables were categorized	n/a
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	14
Discussion			
Key results	18	Summarise key results with reference to study objectives	14-15
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	17-18
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14-18
Generalisability	21	Discuss the generalisability (external validity) of the study results	17
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	19

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.