

Increased IRS2 mRNA Expression in SGA Neonates: PCR Analysis of Insulin/IGF Signaling in Cord Blood

Masanobu Fujimoto,¹ Yuki Kawashima Sonoyama,¹ Kenji Fukushima,¹
Aya Imamoto,¹ Fumiko Miyahara,¹ Naoki Miyahara,¹ Rei Nishimura,¹
Yuko Yamada,¹ Mazumi Miura,¹ Kaori Adachi,² Eiji Nanba,² Keiichi Hanaki,³
and Susumu Kanzaki¹

¹Division of Pediatrics and Perinatology, Tottori University Faculty of Medicine, Yonago, Japan 683-8504; ²Division of Functional Genomics, Research Center for Bioscience and Technology, Tottori University, Yonago, Japan 683-8504; and ³Department of Women's and Children's Family Nursing, Tottori University Faculty of Medicine, Yonago, Japan 683-8504

Context: Hypoglycemia is the most common metabolic problem among small-for-gestational-age (SGA) neonates. However, the pathological mechanism and insulin/insulin-like growth factor (IGF) signaling axis in neonates remain unknown.

Objective: To determine the insulin/IGF axis in neonates, we analyzed the messenger RNA (mRNA) expression of insulin/IGF signaling in fetal umbilical cord blood.

Setting: The Perinatal Medical Center of Tottori University Hospital.

Participants: Fifty-two [42 appropriate-for-gestational-age (AGA) and 10 SGA] neonates.

Interventions: Immediately collected cord blood was placed into a PAXgene Blood RNA Tube. Total RNA from the blood was purified using reagents provided in the PAXgene Blood RNA Kit within 4 days, and reverse transcription polymerase chain reaction (PCR) was performed.

Main Outcome Measure: Quantitative real-time PCR analysis was applied to evaluate the mRNA expression of insulin receptor (*INSR*), IGF-I receptor (*IGF1R*), insulin receptor substrate 1 (*IRS1*), *IRS2*, and glucose transporters (*SLC2A2* and *SLC2A4*). *β-Actin* was used as a control gene.

Results: Serum glucose and IGF-I levels in SGA neonates were significantly lower. The cord serum insulin levels were similar between AGA and SGA neonates. The *IRS2* mRNA expression was significantly higher in SGA than in AGA neonates ($P < 0.05$). The *IRS2* mRNA expression was significantly higher in hypoglycemic SGA neonates than in normoglycemic SGA neonates.

Conclusions: We determined that intrauterine growth restriction induces increased *IRS2* mRNA expression in cord blood, without hyperinsulinemia. The increased expression of *IRS2* mRNA might be associated with abnormal glucose metabolism in SGA neonates. Our findings might lead to the elucidation of abnormal glucose metabolism in SGA neonates.

Copyright © 2017 Endocrine Society

This article has been published under the terms of the Creative Commons Attribution Non-Commercial, No-Derivatives License (CC BY-NC-ND; <https://creativecommons.org/licenses/by-nc-nd/4.0/>).

Freeform/Key Words: insulin/IGF signaling, *IRS2*, quantitative real-time PCR analysis, small-for-gestational age

Abbreviations: AGA, appropriate-for-gestational-age; BW, birth weight; IGF, insulin-like growth factor; IGF1R, insulin-like growth factor-I receptor; *INSR*, insulin receptor; IR, insulin receptor; IRS, insulin receptor substrate; mRNA, messenger RNA; PCR, polymerase chain reaction; SDS, standard deviation score; SGA, small-for-gestational-age.

Insulin and insulin-like growth factor (IGF) signaling contribute to glucose metabolism and growth in a wide range of mammals [1–3]. IGF-I and insulin bind to both the IGF-I receptor (IGF1R) and insulin receptor (INSR) to activate the phosphorylation of IGF1R and INSR and also induce the phosphorylation of IR substrate (IRS), which is the docking protein for both INSR and IGF1R. Phosphorylated IRS proteins act as docking sites for several intracellular proteins such as Grb2, NcK, and the regulatory subunit p85 of phosphatidylinositol 3-kinase, which mediate the different actions of insulin and IGF1 [1]. There are six types of IRS (IRS1 to IRS6), and IRS1 and IRS2 are considered critical intracellular proteins for IGF/insulin signaling [1–3]. Numerous molecular studies have revealed that abnormality of the insulin/IGF signaling pathway induces insulin resistance and type 2 diabetes, especially in obese adults [4–6]. Specifically, obesity promotes a state of chronic low-grade inflammation attributed to the release of free fatty acids, glycerol, hormones (e.g., leptin, adiponectin, and endothelin-1), and proinflammatory cytokines [e.g., tumor necrosis factor α , interleukin-1 β , and interleukin-6] from the adipose tissue; these events negatively regulate IRS kinase and induce abnormal signaling, resulting in insulin resistance.

Small-for-gestational-age (SGA) birth induces hypoglycemia in neonates and is associated with postnatal insulin resistance and type 2 diabetes in adults [7–9]. Hypoglycemia in SGA neonates is caused by inadequate glycogen stores, rapid disappearance rates of glucose, and higher insulin sensitivity [10–12]. Furthermore, postnatal insulin resistance in adult patients born as SGA neonates is associated with rapid postnatal weight gain [9, 13, 14] or decreased β -cell growth due to undernutrition [15–17]. Although these findings suggest abnormal insulin/IGF signaling in SGA neonates, the detailed mechanisms of both hypoglycemia and insulin resistance associated with SGA remain unclear. Real-time polymerase chain reaction (PCR) analysis is a useful tool for the detection of even small amounts of insulin/IGF signaling–related genes from cells or tissues. Several reports on insulin/IGF signaling have examined the placenta of SGA neonates using real-time PCR analysis of insulin/IGF signaling–related genes to determine insulin/IGF signaling abnormalities in the fetus [18, 19]; however, real-time PCR analysis of insulin/IGF signaling–related genes from SGA neonates has not been performed using cord blood, which better reflects the neonate status.

This is, to our knowledge, the first study to perform a quantitative real-time PCR analysis for evaluating the messenger RNA (mRNA) expression of insulin/IGF signaling genes, *IGF1R*, insulin receptor (*INSR*), *IRS1*, *IRS2*, and glucose transporters 2 and 4 (*SLC2A2* and *SLC2A4*, respectively) in umbilical cord blood.

1. Materials and Methods

A. Subjects and Measurement

This study initially included neonates who were born between September 2015 and July 2016 at the Perinatal Medical Center of Tottori University Hospital. We performed this procedure after obtaining consent from their mothers. The inclusion criteria for neonates were term birth (gestational age at birth: 37 to 41 weeks), birth weight (BW) and length <90th percentile based on Japanese neonatal anthropometric charts for gestational age, sex, and the mother's history of childbirth [20]. The exclusion criteria for neonates were preterm birth (gestational age \leq 36 weeks), birth with asphyxia, and birth to mothers with gestational diabetes mellitus diagnosed based on a 75-g oral glucose tolerance test and treated with betamethasone, insulin, and antidiabetic drugs. The neonates of mothers with underlying diseases [hyperthyroidism without antithyroid drugs (n = 1) and hypothyroidism treated with levothyroxine (25 μ g/d; n = 1)] were included. Neonates with malformations or genetic disorders were excluded. Finally, 57 newborns (28 males and 29 females) were recruited. Seventeen of them, who were full-term appropriate-for-gestational-age (AGA) neonates without hypoglycemia and maternal complications, were used as controls. SGA was defined as less than or equal to -2.0 standard deviations of BW and/or birth length on the chart [20]. We immediately measured the plasma glucose levels and serum levels of insulin and IGF-I from the cord blood (collected from the

Table 1. Maternal and Neonatal Characteristics and Laboratory Data in Cord Blood Samples

	AGA Neonates [Median (IQR)]								
	AGA			Normoglycemia			Hypoglycemia		
	n			n			n		
Maternal characteristics									
Age (y)	42	32	(30.0–36.0)	35	33	(30.8–36.5)	7	31	(27.0–36.0)
Weight (kg)	41	63.0	(56.3–68.9)	35	63.2	(58.3–68.6)	6	63.8	(53.4–68.9)
Delivery									
Cesarean Section (n)			11			8			3
Neonatal characteristics									
Males (n, %)	42	19	(45.2%)	35	16	(45.7%)	7	3	(42.9%)
Gestational age (wk)	42	39.0	(37.4–40.5)	35	39.0	(37.9–40.4)	7	37.1	(37.1–37.4) ^b
BW (kg)	42	2.95	(2.70–3.17)	35	3.06	(2.82–3.16)	7	2.50	(2.34–2.58) ^b
BW SDS	42	0.13	(–0.57 to 0.67)	35	0.19	(–0.43 to 0.74)	7	–0.39	(–1.01 to –0.12) ^b
Birth length (cm)	42	48.1	(46.7–49.1)	35	48.3	(47.5–49.3)	7	45.2	(44.0–45.7) ^b
Birth length SDS	42	–0.39	(–0.89 to 0.17)	35	–0.09	(–0.76 to 0.17)	7	–0.98	(–1.27 to 0.86) ^b
Glucose (mg/dL)	39	82	(53–109)	32	89	(70–113)	7	39	(32–43) ^b
Insulin (μIU/mL)	38	2.4	(1.1–4.9)	31	2.9	(1.2–5.9)	7	2.0	(1.2–2.7)
Amended insulin-glucose ratio (pmol/L)/(mmol/L)	39	6.0	(2.6–19.9)	32	5.0	(2.4–10.3)	7	16.4	(7.8–28.5)
Cord IGF-I (ng/mL)	38	54	(43–70)	31	55	(46–78)	7	44	(40–56)

umbilical vein) at birth. The amended insulin-glucose ratios were calculated using the following formula: insulin (pmol/L)/[glucose (mmol/L) – 1.7 mmol/L]. Hypoglycemia was defined as a plasma glucose level of <50 mg/dL (<2.8 mmol/L) within 48 hours of birth. If hypoglycemia was detected, treatments for hypoglycemia with feeding milk or intravenous glucose infusion were instituted. Neonatal mass screening for metabolic disorders was performed in all neonates, but no neonates with positive screening tests were detected. This study was approved by the Ethics Committee of Tottori University.

B. Quantitative Reverse Transcription PCR

The collected cord blood (from the umbilical vein) was immediately placed into a PAXgene Blood RNA Tube (Becton Dickinson and Company, Franklin Lakes, NJ); total RNA from the umbilical cord blood was purified using the reagents provided in the PAX gene Blood RNA Kit (QIAGEN, Germantown, MD) within 4 days, and reverse transcription PCR was performed. First-strand complementary DNA synthesis was performed as described previously [21]. Quantitative real-time PCR analysis was applied for evaluating the expression of *IGF1R*, *INSR*, *IRS1*, *IRS2*, *SLC2A2*, and *SLC2A4* mRNA using the Applied Biosystems 7900HT Fast Real-Time PCR System (Life Technologies Japan Ltd., Tokyo, Japan) with a TaqMan probe and 2× EXPRESS qPCR Supermix with Premixed ROX (Life Technologies, Carlsbad, CA). *β-Actin* was used as a control gene in AGA neonates with normoglycemia. TaqMan probes 22, 54, 52, 49, 85, 67, and 63 were used to detect *IGF1R*, *INSR*, *IRS1*, *IRS2*, *SLC2A2*, *SLC2A4*, and *β-actin*, respectively.

C. Statistical Analysis

Statistical analysis was performed using R (version 3.2.5) and the R Commander package (R Foundation for Statistical Computing, Vienna, Austria). Differences between groups (Table 1) were assessed using nonparametric tests (Mann-Whitney *U* test) or Fisher's exact test. Moreover, the mRNA levels (Figs. 1 and 2) were compared using a parametric test (Welch *t* test) due to normal distribution. The results in Table 1 are expressed as median and interquartile range, and those in Figs. 1 and 2 are expressed mean ± standard error of the mean. A *P* value of <0.05 was considered to indicate a statistically significant difference.

Table 1. Continued

SGA Neonates [Median (IQR)]										
n	SGA		n	Normoglycemia		n	Hypoglycemia		Power	P Value (AGA vs SGA)
10	35	(32.5–37.3)	3	32	(31.5–33.5)	7	35	(34.5–38.5)	0.14	0.26
8	55.2	(50.3–59.1)	2	49.5	(48.8–50.3)	6	56.1	(54.9–64.0)	0.46	0.06
	6			1			5			0.05 ^a
10	6	(60%)	3	0	(0%)	7	6	(85.7%)		0.49 ^a
10	37.7	(37.4–38.2) ^c	3	37.9	(37.4–38.0)	7	37.6	(37.4–38.3)	0.59	<0.01
10	1.95	(1.90–2.00) ^c	3	1.90	(1.73–1.95)	7	1.96	(1.93–2.09)	1.00	<0.01
10	–2.64	(–2.87 to –2.08) ^c	3	–2.83	(–3.16 to –2.70)	7	–2.37	(–2.79 to –1.79)	1.00	<0.01
10	42.9	(41.9–43.0) ^c	3	42.8	(41.9–42.9)	7	43.0	(41.9–43.5)	0.97	<0.01
10	–2.37	(–2.5 to –2.11) ^c	3	–2.38	(–2.46 to –2.37)	7	–2.12	(–2.60 to –2.08)	1.00	<0.01
10	41	(30–54) ^c	3	60	(58–62)	7	33	(15–42) ^d	0.90	<0.01
10	1.5	(0.8–4.1)	3	1.7	(1.5–2.4)	7	0.9	(0.65–7.2)	0.90	0.49
10	15.2	(6.3–36.7)	3	6.4	(6.1–9.8)	7	35.8	(11.7–52.9)	0.47	0.08
10	20	(15–36) ^c	3	16	(12–20)	7	25	(15–42)	0.89	<0.01

Values are given as median (interquartile range). Normal range of amended insulin-glucose ratio is <53.6.

Abbreviations: IQR, interquartile range.

^a*P* values from Fisher's exact test; other *P* values from Mann-Whitney *U* test.

^b*P* < 0.05 between normoglycemia and hypoglycemia in AGA.

^c*P* < 0.01 between all AGA and SGA.

^d*P* < 0.01 between normoglycemia and hypoglycemia in SGA.

2. Results

We analyzed 57 neonates (28 males and 29 females) who met our criteria (Figure 3). Five neonates were excluded because of gestational diabetes mellitus in the mothers. Fifty-two neonates were eligible: 42 AGA and 10 SGA neonates. The clinical and maternal characteristics of the neonates are shown in Table 1. There was no difference in the maternal age and body weight between the AGA and SGA groups. The median BWs in the AGA and SGA groups were 2950 g (2700 to 3170 g) and 1950 g (1900 to 2000 g) (*P* < 0.01), respectively.

The plasma glucose levels in SGA neonates were significantly lower than those in AGA neonates [SGA median: 41 mg/dL (2.27 mmol/L); AGA median: 82 mg/dL (4.56 mmol/L); *P* < 0.01]. Furthermore, 14 neonates (27% of eligible neonates; AGA, *n* = 7; SGA, *n* = 7) exhibited subsequent hypoglycemia, and the rate of subsequent hypoglycemia was significantly higher in the SGA group than in the AGA group (*P* = 0.003). Although the cord serum IGF-I levels in AGA neonates were significantly higher than those in SGA neonates (SGA median, 20 mg/dL; AGA median, 54 mg/dL; *P* < 0.01), the cord serum insulin levels were similar between the AGA and SGA neonates (*P* = 0.9). The normal amended insulin-glucose ratio should be <53.6 [22, 23]; both groups did not show a significant increase, and there were no significant differences. Four neonates (AGA, *n* = 1; SGA, *n* = 3) displayed relatively elevated insulin levels (>2 μIU/mL) with hypoglycemia. Hyperglycemia was not observed in any of the neonates.

A. Comparison of mRNA Levels Between SGA and AGA Neonates

The mRNA expression of *IRS2* was significantly increased in SGA neonates compared with AGA neonates (*P* = 0.013), but there were no differences in the expression of *IGF1R*, *INSR*, *IRS1*, *SLC2A2*, and *SLC2A4* mRNA (Fig. 2). Furthermore, a simple linear regression analysis was performed to predict the relative expression of *IRS2* mRNA in cord blood based on BW

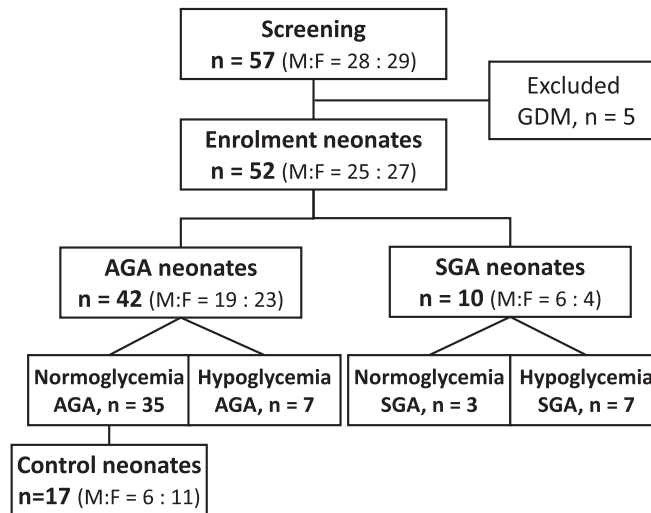


Figure 1. Flow diagram of participants in this study.

standard deviation score (SDS), glucose level, gestational age, and sex (Supplemental Fig. 1), and a significant regression equation ($P = 0.0022$) with an R^2 of 0.176 was obtained only for BW SDS. In the participants, we predicted that the relative expression of *IRS2* mRNA is equal to 1.18 to 0.21 (BW) when the BW is showed in SDS. SGA neonates with subsequent hypoglycemia exhibited significantly higher *IRS2* mRNA expression than did normoglycemic SGA neonates [Fig. 3(A)], whereas the mRNA expression did not significantly differ between the subsequent hypoglycemic and normoglycemic subgroups [Fig. 3(B)] of AGA neonates. However, results of the simple linear regression analysis between the relative expression of *IRS2* mRNA in cord plasma glucose levels were not significant ($P = 0.65$), with an R^2 of 0.0045 (Supplemental Fig. 1). Furthermore, the increased mRNA expression of *SLC2A2* and *SLC2A4* in SGA neonates with subsequent hypoglycemia was not significant, and an increasing trend was observed in these neonates compared with normoglycemic neonates. We also performed the multiple linear regression analysis between three variables [BW (SDS), glucose level, and gestational age] and *IRS2* mRNA separately in male and female. The significant regression equation ($P = 0.033$) with an R^2 of 0.21 was obtained for BW in male, and the

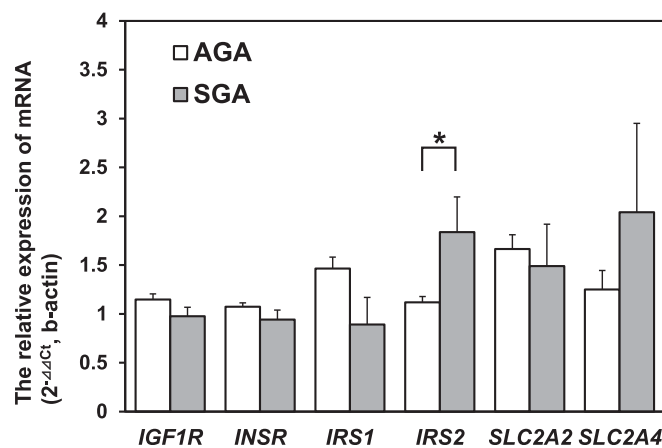


Figure 2. Expression levels of mRNA in cord blood samples from AGA or SGA neonates. *IGF1R*, *INSR*, *IRS1*, *IRS2*, *SLC2A2*, and *SLC2A4* mRNA expression levels in cord blood samples from AGA ($n = 42$) and SGA ($n = 10$) neonates are measured using real-time PCR and analyzed using the $2^{-\Delta\Delta C_t}$ method. $2^{-\Delta\Delta C_t} = 2^{-\Delta C_t} = 2^{-(\Delta C_t - \Delta C_t)}$, for the target mRNA $^{-\Delta C_t}$, for β -actin). The mean expression of β -actin mRNA in AGA neonates with normoglycemia ($n = 17$) is used as a control. The results are presented as mean \pm SE (AGA, $n = 42$; SGA, $n = 10$). * $P < 0.05$ (Welch t -test).

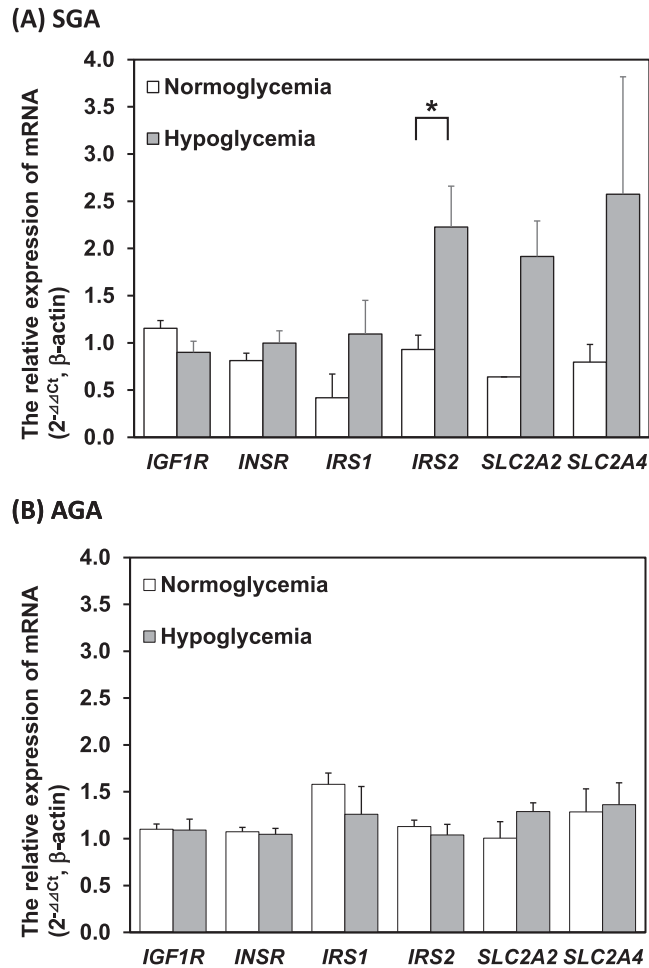


Figure 3. Expression levels of mRNA in cord blood samples from SGA and AGA neonates with normoglycemia or hypoglycemia. (A) *IGF1R*, *INSR*, *IRS1*, *IRS2*, *SLC2A2*, and *SLC2A4* mRNA expression levels in cord blood samples from SGA neonates are grouped according to glucose concentration measured using real-time PCR and are analyzed using the $2^{-\Delta\Delta C_t}$ method (normoglycemia, $n = 3$; hypoglycemia, $n = 7$). (B) *IGF1R*, *INSR*, *IRS1*, *IRS2*, *SLC2A2*, and *SLC2A4* mRNA expression levels in cord blood samples from AGA neonates are grouped according to glucose concentration measured using real-time PCR and are analyzed using the $2^{-\Delta\Delta C_t}$ method (normoglycemia, $n = 31$; hypoglycemia, $n = 7$). $2^{-\Delta\Delta C_t} = 2^{-\Delta C_t} = 2^{-(C_t - C_{\beta-actin})}$, for the target mRNA $-C_t$, for $\beta-actin$). The mean expression of $\beta-actin$ mRNA in AGA neonates with normoglycemia ($n = 17$) is used as a control. The results are presented as mean \pm SEM. * $P < 0.05$ (Welch t -test).

nonsignificant equation ($P = 0.054$) with an R^2 of 0.3 was obtained for BW in female. There is no regression equation between *IRS2* mRNA and all other variables (data not shown).

3. Discussion

In this study, the *IRS2* mRNA expression was significantly higher in the SGA neonates, especially in those with hypoglycemia. Moreover, the cord blood from the SGA neonates showed lower glucose and serum IGF-I levels, without hyperinsulinemia. To the best of our knowledge, this is the first study of *IGF1R*, *INSR*, *IRS1-2*, *SLC2A2*, and *SLC2A4* mRNA in cord blood.

Although insulin and IGF1 play distinct physiological roles in glucose metabolism and growth, both insulin and IGF signals use the same signaling pathways [1, 24]. Furthermore, insulin and IGF1 have both common and complementary effects [25]. Previous animal or

human studies have demonstrated that SGA neonates have lower serum IGF1, insulin, and glucose levels [26–28]. Furthermore, higher insulin sensitivity is related to hypoglycemia in SGA neonates [27, 29]. From these findings, we expected the abnormal expression of mRNA to be related to the insulin/IGF signals of *IGF1R*, *INSR*, *IRS1-2*, *SLC2A2*, and *SLC2A4* in SGA neonates, which were evaluated in this study. We detected lower serum glucose and IGF1 levels in the SGA group, which is consistent with previous reports [26–28]; however, we observed no difference in the expression of *IGF1R*, *INSR*, *SLC2A2*, and *SLC2A4* in the SGA neonates, and the expression of only *IRS2* mRNA was significantly increased in these neonates, especially in those with subsequent hypoglycemia. Given that hyperinsulinemia decreases the expression of *INSR*, *IRS1*, and *IRS2* [30–32], our findings suggest that the SGA neonates did not exhibit hyperinsulinemia, at least in the fetal cord blood.

An explanation for why the cord blood from the SGA neonates showed increased *IRS2* mRNA expression is as follows. *IRS1* and *IRS2* are docking proteins for both IR and IGF1R [1, 31]. The activated phosphorylation of *INSR* and IGF1R by insulin or IGF1 binding induces the phosphorylation of *IRS*, resulting in intracellular signaling, which has an essential role in growth and metabolism [1, 2, 33]. Furthermore, the expression of *Irs2* mRNA and *Irs2* protein was remarkably increased during 24-hour fasting and decreased rapidly after refeeding in normal mice [34]. These findings suggest that the increased expression of *IRS2* mRNA in SGA neonates is caused by fetal undernutrition, and the significant regression equation between the expression of *IRS2* mRNA and BW is consistent with these findings. Furthermore, the upregulation of *IRS2* may play a role in the increased sensitivity to insulin [35]. Mohanty *et al.* [36] revealed that the overexpression of *Irs2* in isolated rat islets significantly increased basal and D-glucose-stimulated insulin levels. These findings also indicate that the considerably increased *IRS2* mRNA expression in the SGA neonates with hypoglycemia in our study might be related to higher insulin sensitivity and hypoglycemia in SGA neonates, which has also been previously reported [27, 29]. Furthermore, the increased mRNA expression of *SLC2A2* and *SLC2A4* in SGA neonates with subsequent hypoglycemia was observed but was not significant. Glucose transporters GLUT2 (*SLC2A2*) and GLUT4 (*SLC2A4*) are thought to be activated by *IRS2* [37, 38]. Accordingly, the increased *IRS2* mRNA expression in SGA with subsequent hypoglycemia might induce the increased mRNA expression of *SLC2A2* and *SLC2A4*. Furthermore, GLUT2 and GLUT4 may be the downstream proteins in *IRS2* signaling, and these finding may reflect the variation in mRNA expression of these proteins; however, further studies are needed.

Moreover, SGA is also associated with postnatal insulin resistance in adults [9, 12, 39], and these conditions are known as the fetal programming of diabetes (Developmental Origins of Health and Disease) [40]. The increased mRNA expression of *IRS2*, which is an important factor for insulin signaling, might be associated with this fetal programming, and we are planning to perform these experiments in infants, children, and adults.

A limitation of this study was the small number of assessed SGA neonates ($n = 10$). We intend to continue this study with more subjects comprising premature infants, neonates, and children.

In conclusion, we determined that SGA is associated with higher *IRS2* mRNA expression without hyperinsulinemia. The increased expression of *IRS2* mRNA might be associated with abnormal glucose metabolism, leading to hypoglycemia and postnatal insulin resistance in SGA neonates. Our study findings might aid in the elucidation of the abnormal glucose metabolism in SGA neonates.

Acknowledgments

We thank the participants in this study and their families, as well as the healthcare professionals involved in the Perinatal Medical Center of Tottori University Hospital and the doctors in the Division of Reproductive-Perinatal Medicine and Gynecologic Oncology, Tottori University Faculty of Medicine, Tottori, Japan.

Financial Support: This work was supported by the Foundation for Growth Science, the Tottori University Faculty of Medicine Alumni Association, and Tottori University Hospital.

Correspondence: Yuki Kawashima Sonoyama, PhD, MD, Division of Pediatrics and Perinatology, Tottori University Faculty of Medicine, 36-1 Nishi-cho, Yonago 683-8504, Japan. E-mail: yuki.kawashima@med.tottori-u.ac.jp.

Disclosure Summary: The authors have nothing to disclose.

References and Notes

1. Boucher J, Kleinridders A, Kahn CR. Insulin receptor signaling in normal and insulin-resistant states. *Cold Spring Harb Perspect Biol.* 2014;**6**(1):6.
2. Adams TE, Epa VC, Garrett TP, Ward CW. Structure and function of the type 1 insulin-like growth factor receptor. *Cell Mol Life Sci.* 2000;**57**(7):1050–1093.
3. Clemmons DR. Metabolic actions of insulin-like growth factor-I in normal physiology and diabetes. *Endocrinol Metab Clin North Am.* 2012;**41**(2):425–443.
4. Brady MJ. IRS2 takes center stage in the development of type 2 diabetes. *J Clin Invest.* 2004;**114**(7):886–888.
5. Saini V. Molecular mechanisms of insulin resistance in type 2 diabetes mellitus. *World J Diabetes.* 2010;**1**(3):68–75.
6. Kahn BB, Flier JS. Obesity and insulin resistance. *J Clin Invest.* 2000;**106**(4):473–481.
7. Collins JE, Leonard JV, Teale D, Marks V, Williams DM, Kennedy CR, Hall MA. Hyperinsulinaemic hypoglycaemia in small for dates babies. *Arch Dis Child.* 1990;**65**(10):1118–1120.
8. Barker DJ, Hales CN, Fall CH, Osmond C, Phipps K, Clark PM. Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. *Diabetologia.* 1993;**36**(1):62–67.
9. Geremia C, Cianfarani S. Insulin sensitivity in children born small for gestational age (SGA). *Rev Diabet Stud.* 2004;**1**(2):58–65.
10. McGowan JE. Neonatal hypoglycemia. *Pediatrics.* 1999;**20**:e6–e15.
11. Wiswell TE, Cornish JD, Northam RS. Neonatal polycythemia: frequency of clinical manifestations and other associated findings. *Pediatrics.* 1986;**78**(1):26–30.
12. Hawdon JM, Weddell A, Aynsley-Green A, Ward Platt MP. Hormonal and metabolic response to hypoglycaemia in small for gestational age infants. *Arch Dis Child.* 1993;**68**(3):269–273.
13. Okada T, Takahashi S, Nagano N, Yoshikawa K, Usukura Y, Hosono S. Early postnatal alteration of body composition in preterm and small-for-gestational-age infants: implications of catch-up fat. *Pediatr Res.* 2015;**77**(1-2):136–142.
14. Cianfarani S, Germani D, Branca F. Low birthweight and adult insulin resistance: the “catch-up growth” hypothesis. *Arch Dis Child Fetal Neonatal Ed.* 1999;**81**(1):F71–F73.
15. Garofano A, Czernichow P, Bréant B. In utero undernutrition impairs rat beta-cell development. *Diabetologia.* 1997;**40**(10):1231–1234.
16. Garofano A, Czernichow P, Bréant B. Effect of ageing on beta-cell mass and function in rats malnourished during the perinatal period. *Diabetologia.* 1999;**42**(6):711–718.
17. Bouwens L, Rooman I. Regulation of pancreatic beta-cell mass. *Physiol Rev.* 2005;**85**(4):1255–1270.
18. Iñiguez G, González CA, Argandoña F, Kakarić E, Johnson MC, Cassorla F. Expression and protein content of IGF-I and IGF-I receptor in placentas from small, adequate and large for gestational age newborns. *Horm Res Paediatr.* 2010;**73**(5):320–327.
19. Hiden U, Glitzner E, Hartmann M, Desoye G. Insulin and the IGF system in the human placenta of normal and diabetic pregnancies. *J Anat.* 2009;**215**(1):60–68.
20. Itabashi K, Miura F, Uehara R, Nakamura Y. New Japanese neonatal anthropometric charts for gestational age at birth. *Pediatr Int.* 2014;**56**(5):702–708.
21. Fujimoto M, Kawashima Sonoyama Y, Hamajima N, Hamajima T, Kumura Y, Miyahara N, Nishimura R, Adachi K, Nanba E, Hanaki K, Kanzaki S. Heterozygous nonsense mutations near the C-terminal region of IGF1R in two patients with small-for-gestational-age-related short stature. *Clin Endocrinol (Oxf).* 2015;**83**(6):834–841.
22. Turner RC, Oakley NW, Nabarro JD. Control of basal insulin secretion, with special reference to the diagnosis of insulinomas. *BMJ.* 1971;**2**(5754):132–135.
23. Nauck MA, Meier JJ. Diagnostic accuracy of an “amended” insulin-glucose ratio for the biochemical diagnosis of insulinomas. *Ann Intern Med.* 2012;**157**(11):767–775.
24. Siddle K. Molecular basis of signaling specificity of insulin and IGF receptors: neglected corners and recent advances. *Front Endocrinol (Lausanne).* 2012;**3**:34.

25. Boucher J, Tseng YH, Kahn CR. Insulin and insulin-like growth factor-1 receptors act as ligand-specific amplitude modulators of a common pathway regulating gene transcription. *J Biol Chem*. 2010;**285**(22):17235–17245.
26. Karamizadeh Z, Saki S, Kashef S, Saki F. Comparison of umbilical cord and maternal serum levels of IGF-1, leptin and cortisol in appropriate for gestational age and small for gestational age neonates. *Int J Endocrinol Metab*. 2008;**6**:89–94.
27. Bazaes RA, Salazar TE, Pittaluga E, Peña V, Alegría A, Iñiguez G, Ong KK, Dunger DB, Mericq MV. Glucose and lipid metabolism in small for gestational age infants at 48 hours of age. *Pediatrics*. 2003;**111**(4 Pt 1):804–809.
28. Chiesa C, Osborn JF, Haass C, Natale F, Spinelli M, Scapillati E, Spinelli A, Pacifico L. Ghrelin, leptin, IGF-1, IGFBP-3, and insulin concentrations at birth: is there a relationship with fetal growth and neonatal anthropometry? *Clin Chem*. 2008;**54**(3):550–558.
29. Limesand SW, Rozance PJ, Smith D, Hay WW, Jr. Increased insulin sensitivity and maintenance of glucose utilization rates in fetal sheep with placental insufficiency and intrauterine growth restriction. *Am J Physiol Endocrinol Metab*. 2007;**293**(6):E1716–E1725.
30. Huang X, Vaag A, Hansson M, Groop L. Down-regulation of insulin receptor substrates (IRS)-1 and IRS-2 and Src homologous and collagen-like protein Shc gene expression by insulin in skeletal muscle is not associated with insulin resistance or type 2 diabetes. *J Clin Endocrinol Metab*. 2002;**87**(1):255–259.
31. Taniguchi CM, Emanuelli B, Kahn CR. Critical nodes in signalling pathways: insights into insulin action. *Nat Rev Mol Cell Biol*. 2006;**7**(2):85–96.
32. Hirashima Y, Tsuruzoe K, Kodama S, Igata M, Toyonaga T, Ueki K, Kahn CR, Araki E. Insulin down-regulates insulin receptor substrate-2 expression through the phosphatidylinositol 3-kinase/Akt pathway. *J Endocrinol*. 2003;**179**(2):253–266.
33. Aguirre GA, De Ita JR, de la Garza RG, Castilla-Cortazar I. Insulin-like growth factor-1 deficiency and metabolic syndrome. *J Transl Med*. 2016;**14**:3.
34. Kubota N, Kubota T, Itoh S, Kumagai H, Kozono H, Takamoto I, Mineyama T, Ogata H, Tokuyama K, Ohsugi M, Sasako T, Moroi M, Sugi K, Kakuta S, Iwakura Y, Noda T, Ohnishi S, Nagai R, Tobe K, Terauchi Y, Ueki K, Kadowaki T. Dynamic functional relay between insulin receptor substrate 1 and 2 in hepatic insulin signaling during fasting and feeding. *Cell Metab*. 2008;**8**(1):49–64.
35. Dominici FP, Hauck S, Argentino DP, Bartke A, Turyn D. Increased insulin sensitivity and upregulation of insulin receptor, insulin receptor substrate (IRS)-1 and IRS-2 in liver of Ames dwarf mice. *J Endocrinol*. 2002;**173**(1):81–94.
36. Mohanty S, Spinass GA, Maedler K, Zuellig RA, Lehmann R, Donath MY, Trüb T, Niessen M. Overexpression of IRS2 in isolated pancreatic islets causes proliferation and protects human beta-cells from hyperglycemia-induced apoptosis. *Exp Cell Res*. 2005;**303**(1):68–78.
37. Park S, Dong X, Fisher TL, Dunn S, Omer AK, Weir G, White MF. Exendin-4 uses Irs2 signaling to mediate pancreatic β cell growth and function. *J Biol Chem*. 2006;**281**(2):1159–1168.
38. Santamaria B, Marquez E, Lay A, Carew RM, González-Rodríguez Á, Welsh GI, Ni L, Hale LJ, Ortiz A, Saleem MA, Brazil DP, Coward RJ, Valverde ÁM. IRS2 and PTEN are key molecules in controlling insulin sensitivity in podocytes. *Biochim Biophys Acta*. 2015;**1853**(12):3224–3234.
39. Putzker S, Bechtold-Dalla Pozza S, Kugler K, Schwarz HP, Bonfig W. Insulin resistance in young adults born small for gestational age (SGA). *J Pediatr Endocrinol Metab*. 2014;**27**(3-4):253–259.
40. Gluckman PD, Hanson MA, Cooper C, Thornburg KL. Effect of in utero and early-life conditions on adult health and disease. *N Engl J Med*. 2008;**359**(1):61–73.