

## Cord Blood from SGA Preterm Infants Exhibits Increased GLUT4 mRNA Expression

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### ABSTRACT

**Background** Insulin and insulin-like growth factor (IGF) signaling plays an important role in prenatal and postnatal growth and glucose metabolism. Both small-for-gestational age (SGA) and preterm infants have abnormal growth and glucose metabolism. However, the underlying mechanism remains unknown. Recently, we showed that term SGA infants have abnormal insulin/IGF signaling in cord blood. In this study, we examined whether preterm infants show similar aberrations in cord blood insulin/IGF signaling.

**Methods** A total of 41 preterm cord blood samples were collected. Blood glucose, insulin, IGF-1, and C-peptide concentrations were measured, and mRNA expression of *IGF1R*, *INSR*, *IRS1*, *IRS2*, and *SLC2A4* (*i.e.*, GLUT4) was analyzed by quantitative reverse-transcription PCR.

**Results** This study included 34 appropriate-for-gestational age (AGA) and 7 SGA preterm neonates. No hyperinsulinemia or any differences in *IGF1R* or *INSR* mRNA expression were detected between the two groups. However, GLUT4 mRNA levels were increased in preterm SGA. Moreover, the expression level in hypoglycemic preterm SGA was significantly higher than that in hypoglycemic preterm AGA. *IRS2* mRNA expression did not show a statistically significant difference between preterm SGA and AGA neonates.

**Conclusion** SGA preterm birth does not induce hyperinsulinemia; however, it modifies insulin/IGF signaling components such as GLUT4 in umbilical cord blood. Our study suggests that prematurity or adaptation to malnutrition alters the insulin/IGF signaling pathway.

**Key words** hypoglycemia; insulin/IGF-1 signaling; preterm; small-for-gestational age

Insulin and insulin-like growth factor (IGF) signaling play an important role in prenatal and postnatal growth and glucose metabolism.<sup>1–3</sup> IGF-1 and insulin bind to both IGF-1 receptor (IGF-1R) and insulin receptor (INSR) to activate their phosphorylation and also

induce phosphorylation of insulin receptor substrate (IRS). Phosphorylated IRS proteins act as docking sites for several intracellular proteins and promote glucose transporter 4 (GLUT4) translocation from vesicles to the cell membrane, which explains the difference between insulin and IGF-1 actions.<sup>1</sup> IRS1 and IRS2 are considered critical intracellular proteins for insulin/IGF signaling.<sup>1–3</sup> Numerous molecular studies have revealed that abnormalities in the insulin/IGF signaling pathway induce insulin resistance and type 2 diabetes.<sup>4–6</sup> For example, increased release of free fatty acids, glycerol, hormones (*e.g.*, leptin, adiponectin, and endothelin-1),<sup>3, 4, 6, 7</sup> and pro-inflammatory cytokines (*e.g.*, tumor necrosis factor  $\alpha$ , interleukin-1 $\beta$ , and interleukin-6) from adipose tissue in obesity, affects IRS kinase and induces abnormal signaling, resulting in insulin resistance.<sup>8–10</sup> However, there are few reports on the insulin/IGF-1 signaling in neonates.

Furthermore, small-for-gestational age (SGA) neonates have abnormal growth and glucose metabolism, such as hypoglycemia,<sup>11</sup> which is occasionally associated with hyperinsulinemia, postnatal insulin resistance, and growth failure in adulthood.<sup>12–18</sup> In addition, preterm infants also have been shown to demonstrate abnormal growth and glucose metabolism analogous to term SGA infants.<sup>19–21</sup> However, the mechanisms underlying these effects are still unclear, and the modes both “SGA” and “preterm” impact insulin/IGF signaling pathway remain unknown.

Monocytes and erythrocytes are known to express insulin and IGF-1 receptors.<sup>22</sup> In very early studies, insulin/IGF signaling was studied using human circulating blood cells.<sup>23</sup> Accordingly, we have

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Received 2020 November 17

Accepted 2020 December 21

Online published 2021 January 20

Abbreviations: AGA, appropriate-for-gestational age; GLUT4, Glucose transporter 4; IGF, insulin-like growth factor; IGF-1R, IGF-1 receptor; INSR, insulin receptor; IRS, insulin receptor substrate; SGA, small-for-gestational age; SLC2A4, solute carrier family 2 member 4

previously studied to evaluate how SGA impact insulin/IGF signaling pathway, and have shown that term SGA infants have abnormal insulin/IGF signaling as higher *IRS2* mRNA levels were detected in SGA subjects.<sup>24</sup> In this study, we investigated insulin/IGF signaling in cord blood from preterm infants to determine how preterm and SGA impact insulin/IGF signaling pathway.

## MATERIALS AND METHODS

### Subjects and measurement

This study initially included all preterm neonates (gestational age < 37 weeks) who were born between June 2018 and July 2019 at the Perinatal Medical Center of Tottori University Hospital. We performed all the procedures after obtaining the consent from the mothers. We then excluded neonates who met the following criteria: Apgar score < 5 at 5 min, neonates with congenital disorders, malformations, or genetic disorders, and those born from mothers having diabetes or with gestational diabetes mellitus with any treatment. We also obtained information of the mothers and neonates from electronic medical records. We then defined SGA neonates as those with birth weight and birth length below the 10<sup>th</sup> percentile of the local sex-specific distribution for gestational age, and appropriate-for-gestational age (AGA) neonates as those with birth weight and/or birth length between the 25<sup>th</sup> and 75<sup>th</sup> percentile of the local sex-specific distribution for gestational age, based on the Japanese neonatal anthropometric charts for gestational age, sex, and the mother's history of child-birth.<sup>25</sup> Accordingly, 7 SGA and 34 AGA neonates were included; AGA neonates were considered as controls. We immediately measured the levels of plasma glucose, serum insulin, C-peptide, and IGF-1 using 5-8 mL of cord blood collected from the umbilical vein at birth as described previously.<sup>24</sup> Hypoglycemia was defined as plasma glucose levels < 50 mg/dL (< 2.8 mmol/L). When hypoglycemia was detected, treatment was instituted by feeding milk or by intravenous glucose infusion. None of the neonates tested positive for neonatal mass screening tests for inborn errors of metabolism. This study was approved by the Ethical Review Board, Tottori University School of Medicine, Japan (No. 17A056). This investigation was conducted according to the principles mentioned in the Declaration of Helsinki. Patients information and identity were kept anonymous prior to conducting mRNA analysis.

### Quantitative RT-PCR

Quantitative RT-PCR was performed as described previously.<sup>24</sup> Briefly, cord blood samples were collected immediately after birth into PAX gene® Blood RNA

Tubes (Becton, Dickinson and Company, UK), and total RNA from umbilical cord blood was purified using the reagents provided in the PAX gene® Blood RNA Kit (QIAGEN, Germany) within 5 days, followed by reverse transcription. First-strand cDNA synthesis was performed as described previously.<sup>24</sup> Quantitative real-time PCR analysis was applied to evaluate expression of *IGF1R*, *INSR*, *IRS1*, *IRS2*, and glucose transporter 4 (*SLC2A4*, known as GLUT4) mRNA by using ViiA 7® Real-Time PCR System (Thermo Fisher Scientific Inc., MA) with Universal Probe Library (Roche Molecular Systems, Inc., CA) and TaqMan Gene Expression Assay (Thermo Fisher Scientific Inc., MA). In this study, *SLC2A2* mRNA, one of the targets of our previous study, could not be analyzed due to technical issues. The mean mRNA level of  $\beta$ -actin from all preterm AGA neonates with normoglycemia was used as the control. Universal Probe Library® probes #22, #54, #49, #67, and #63 were used to detect *IGF1R*, *INSR*, *IRS1*, *IRS2*, *SLC2A4*, and  $\beta$ -actin expression, respectively. TaqMan Gene Expression Assay® (Hs00178563\_ml) was used for *IRS1*. We calculated relative mRNA expression levels using the  $2^{-\Delta\Delta C_t}$  method. The quality of samples was assessed using RNA integrity number (RIN).<sup>26</sup> RINs were 8 or greater in 30 samples, 7 in 10 samples, and 5 in 1 sample.

### Statistical analysis

Statistical analysis was performed using the statistical software 'EZR' (Easy R, version 1.40),<sup>27</sup> which is based on R (version 3.5.2) and R commander (version 2.5-1, R Foundation for Statistical Computing, Vienna, Austria). Differences between groups (Table 1) were assessed using Welch's tests. The correlations of each item with gestational age or birth weight were analyzed using the Spearman rank correlation coefficient (Tables 2, 3 and Figs. 1, 2). Welch's tests were used again to examine the differences of mRNA expressions between the groups (Fig. 3). We also used the Kruskal-Wallis test to compare the differences among the combination of hypoglycemia/normoglycemia with AGA/SGA. As a post-hoc test, Steel-Dwass test were additionally performed to compare for each pair. (Table 4 and Fig. 4). The results in Tables 1 and 4 are expressed as median and interquartile range, and those in Figs. 3 and 4 are expressed as mean  $\pm$  standard error of the mean. A *p*-value < 0.05 was considered to indicate a statistically significant difference.

## RESULTS

### Patients background

There were 87 preterm infants born during the

**Table 1. Characteristics of preterm neonates**

	Total <i>n</i> = 41	AGA <i>n</i> = 34	SGA <i>n</i> = 7	<i>P</i> -value
<b>Maternal</b>				
Age (years)	30 (29 to 34)	30.5 (29 to 34)	30 (29.5 to 35)	0.44
BMI (kg/cm <sup>2</sup> )	24.2 (22.8 to 25.4)	24.5 (23.0 to 25.4)	24.9 (21.7 to 25.6)	0.61
Vaginal delivery ( <i>n</i> , %)	17 (41)	17 (50)	0 (0)	<b>&lt; 0.05</b>
<b>Neonatal</b>				
Male ( <i>n</i> , %)	29 (71)	25 (74)	4 (57)	0.4
Gestational age (weeks)	34 (34 to 36)	34 (34 to 36)	34 (32 to 35)	0.48
Birth weight (gram)	2,105 (1,699 to 2,331)	2,163 (1,877 to 2,370)	1,551 (1,346 to 1,707)	<b>&lt; 0.01</b>
Birth weight SDS	-0.66 (-1.23 to -0.06)	-0.48 (-0.69 to 0.01)	-1.64 (-2.39 to -1.46)	<b>&lt; 0.01</b>
Birth length (cm)	43.5 (40 to 45.5)	44 (42 to 45.5)	38 (35 to 39.5)	<b>&lt; 0.01</b>
Birth length SDS	-0.89 (-1.23 to -0.14)	-0.56 (-1.09 to 0.08)	-2.54 (-2.99 to -2.41)	<b>&lt; 0.01</b>
Singleton ( <i>n</i> , %)	25 (61)	22 (65)	3 (43)	0.4
Apgar score 1 min	7 (5 to 8)	8 (7 to 8)	4.5 (4 to 6)	0.09
Apgar score 5 min	9 (8 to 9)	9 (8 to 9)	8.5 (7.5 to 9)	0.65
Hypoglycemia ( <i>n</i> , %)	19 (46)	15 (44)	4 (57)	0.69
Glucose (mg/dL)	51 (41 to 61)	51 (44.25 to 62.5)	49 (40.5 to 58.5)	0.7
Insulin (μU/mL)	2.10 (1.28 to 3.80)	2.34 (1.34 to 4.66)	1.2 (0.82 to 1.25)	<b>&lt; 0.05</b>
IGF-1 (ng/mL)	42.7 (25.4 to 56.7)	47.2 (29.85 to 59.25)	27.6 (16 to 34.5)	<b>&lt; 0.05</b>
C-Peptide (ng/mL)	0.7 (0.5 to 0.9)	0.65 (0.5 to 0.98)	0.7 (0.65 to 0.73)	0.94

Values are given as median (interquartile range) unless otherwise noted. Significant differences ( $P < 0.05$ ) are marked in bold. IGF-1, insulin-like growth factor-1.

**Table 2. Spearman correlation between gestational age and each mRNA expression**

	Correlation with <i>IGF1R</i>		Correlation with <i>INSR</i>		Correlation with <i>IRS1</i>		Correlation with <i>IRS2</i>		Correlation with GLUT4	
	$r_s$	<i>P</i>	$r_s$	<i>P</i>	$r_s$	<i>P</i>	$r_s$	<i>P</i>	$r_s$	<i>P</i>
AGA	0.16	0.38	0.02	0.93	-0.16	0.38	-0.002	1	-0.13	0.46
SGA	-0.4	0.37	-0.09	0.85	-0.84	<b>&lt; 0.05</b>	0.164	0.73	-0.13	0.79
Overall	-0.04	0.8	-0.04	0.79	-0.26	0.09	-0.04	0.82	-0.17	0.28

The significant correlations ( $P < 0.05$ ) are marked in bold.

**Table 3. Spearman correlation between birth weight SDS and each mRNA expression**

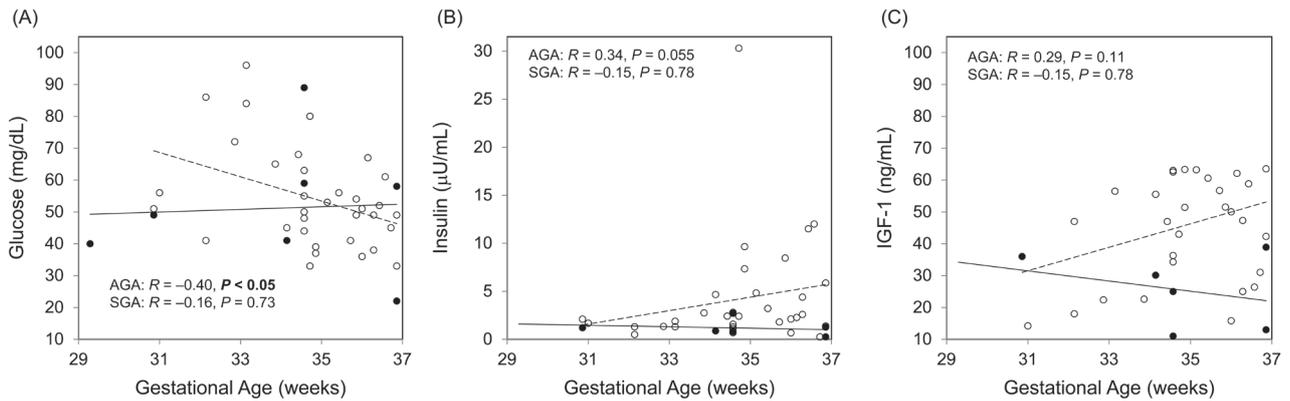
	Correlation with <i>IGF1R</i>		Correlation with <i>INSR</i>		Correlation with <i>IRS1</i>		Correlation with <i>IRS2</i>		Correlation with GLUT4	
	$r_s$	<i>P</i>	$r_s$	<i>P</i>	$r_s$	<i>P</i>	$r_s$	<i>P</i>	$r_s$	<i>P</i>
	-0.20	0.22	-0.15	0.35	0.06	0.73	-0.26	0.10	-0.44	<b>&lt; 0.01</b>

Significant correlations ( $P < 0.05$ ) are marked in bold.

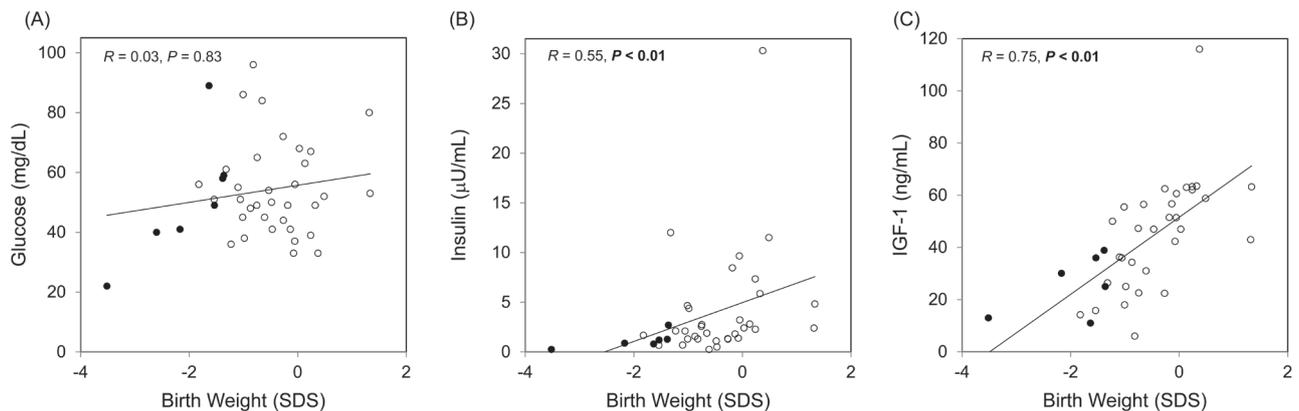
study period. Among them, the parents of 28 did not participate in this study. Among the remaining 59 infants, 9 had insufficient cord blood. We were able to collect sufficient cord blood from the other 50 preterm neonates, but 9 of them were excluded because of

congenital anomalies and improper specimen preparation. Therefore, the analysis was performed on 41 neonatal cord blood samples.

The clinical characteristics and maternal information are shown in Table 1. All *p*-values express the



**Fig. 1.** Spearman correlation between gestational age and (A) glucose, (B) insulin, or (C) IGF-1. White circles and the dashed line denote AGA; black circles and the solid line denote SGA. Significant correlations ( $P < 0.05$ ) are marked in bold. AGA, appropriate-for-gestational age; IGF-1, insulin-like growth factor-1; SGA, small-for-gestational age.



**Fig. 2.** Spearman correlation between birth weight SDS and (A) glucose, (B) insulin, or (C) IGF-1. White circles and the dashed line denote AGA; black circles and the solid line denote SGA. Significant correlations ( $P < 0.05$ ) are marked in bold. AGA, appropriate-for-gestational age; IGF-1, insulin-like growth factor-1; SGA, small-for-gestational age.

difference between AGA and SGA using Welch *t*-test. In total, 41 newborns (29 males and 12 females) were included. They were all born with gestational age between 29 and 36 weeks, and were all classified as having low birth weight. Of these, 34 (83%) were AGA neonates, and the remaining 7 (17%) were SGA neonates. There was no difference in the maternal age and body-mass index between the AGA and SGA groups. The median gestational age between the AGA and SGA groups was not significantly different. As expected, birth weight was significantly lower in the SGA group ( $P < 0.01$ ). The median plasma glucose levels between the 2 groups were not significantly different (AGA: 51 mg/dL, SGA: 49 mg/dL;  $P = 0.7$ ); hypoglycemia occurred in 15 AGA neonates (44%) and in 4 SGA neonates (57%), with no statistical difference ( $P = 0.69$ ). Furthermore, the serum insulin level was significantly lower ( $P = 0.02$ ) in the SGA compared to the AGA. Serum IGF-1 levels were

significantly lower ( $P = 0.04$ ) in the SGA subjects. There was no significant difference in the serum C-peptide levels between the 2 groups ( $P = 0.94$ ). Additionally, none of the subjects suffered from hyperinsulinemic hypoglycemia during the neonatal period.

#### Correlation between mRNA expression in cord blood and gestational age or clinical indicator

Figure 1 shows the correlation between gestational age and each biochemical test result. The glucose (A) level in AGA was positively correlated with gestational age. With regard to mRNA expression (Table 2), *IRSI* mRNA in the SGA showed a negative correlation with gestational age. The mRNA expression of other genes did not correlate with gestational age.

**Table 4. Comparison of biomolecules and birth history among the combinations of AGA/SGA and normoglycemia/hypoglycemia**

	Total	AGA		SGA		P-value
		Normoglycemia	Hypoglycemia	Normoglycemia	Hypoglycemia	
<i>N</i> (n, %)	41 (100)	19 (46)	15 (37)	3 (7)	4 (10)	
Gestational age (weeks)	34 (34 to 36)	34 (33 to 35)	35 (34 to 36)	34 (34 to 35)	32 (29.8 to 34.5)	0.14
Birth weight SDS	-0.66 (-1.23 to -0.06)	-0.53 (-1.03 to 0.08)	-0.27 (-0.81 to -0.07)	-1.38 (-1.51 to -1.37)	-2.39 (-2.83 to -2.01)	<b>&lt; 0.01</b>
Insulin (μU/mL)	2.10 (1.28 to 3.80)	2.19 (1.31 to 2.79)	2.58 (1.48 to 6.60)	1.27 (1.04 to 1.99)	0.87 (0.56 to 1.04)	0.08
IGF-1 (ng/mL)	42.7 (25.4 to 56.7)	36.3 (22.4 to 58.8)	51.4 (44.7 to 59.6)	25.0 (18.0 to 38.9)	30.1 (21.6 to 33.1)	<b>&lt; 0.05</b>
C-peptide (ng/mL)	0.7 (0.5 to 0.9)	0.7 (0.5 to 1.05)	0.6 (0.55 to 0.95)	0.75 (0.73 to 0.78)	0.6 (0.55 to 0.65)	0.89

Values are given as median (interquartile range) unless otherwise noted. Significant correlations ( $P < 0.05$ ) are marked in bold. IGF-1, insulin-like growth factor-1.

### Correlation between mRNA expression in cord blood and birth weight or clinical indicator

Birth weight standard deviation score (SDS) was positively correlated with insulin and IGF-1 ( $r_s = 0.55$ ;  $P < 0.01$ ,  $r_s = 0.75$ ;  $P < 0.01$ , respectively) (Figs. 2A–C). The mRNA expression analysis revealed a negative correlation between birth weight SDS and GLUT4 mRNA ( $r_s = -0.44$ ;  $P < 0.01$ ) (Table 3). *IRS2* mRNA was not significantly correlated with birth weight SDS.

### Comparison of mRNA expression levels in cord blood cells between AGA and SGA preterm infants

As shown in Fig. 3, no difference was observed in the mRNA expression levels of *IGF1R* and *INSR* between the SGA and AGA neonates. On the other hand, GLUT4 mRNA expression, which showed no significant increase in the term study, was significantly increased by 1.7 times in the SGA preterm neonates compared to that in the AGA preterm neonates ( $P < 0.01$ ). Increased *IRS2* mRNA expression was observed in SGA neonates but was not statistically significant ( $P = 0.20$ ), unlike in the term study.

### Effects of hypoglycemia on the mRNA expression in cord blood

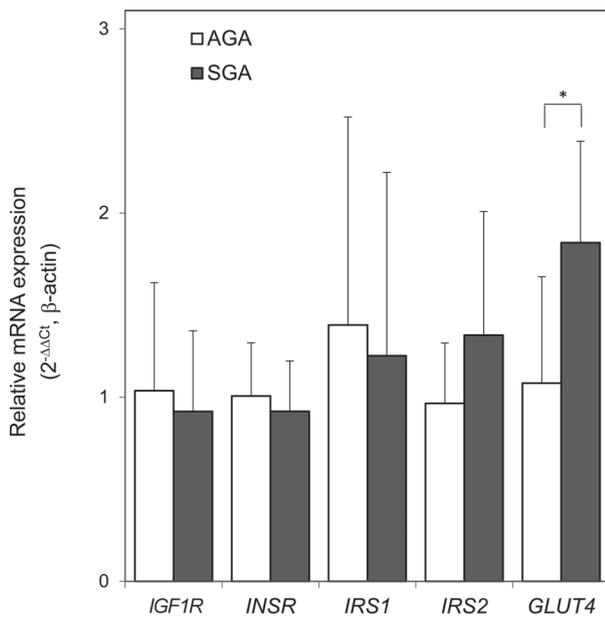
We next utilized the Kruskal-Wallis test to compare among the 4 groups: AGA with normoglycemia, AGA with hypoglycemia, SGA with normoglycemia, and SGA with hypoglycemia, and Steel-Dwass test to compare each pair. The characteristics of these groups are listed in Table 4, and a comparison of each mRNA examined is shown in Fig. 4. *P*-values in Table 4 express the difference among the 4 groups. There was no increase in insulin level in the hypoglycemic SGA cord blood. IGF-1 levels exhibited a significant difference

among the four groups ( $P = 0.04$ ) but showed no difference between each pair in the post-hoc test. The birth weight SDS in the SGA with hypoglycemia group was lower than that without hypoglycemia, but this difference was not significant in the Steel-Dwass test ( $P = 0.29$ ). However, GLUT4 mRNA (Fig. 4E) levels in the SGA neonates with hypoglycemia were significantly higher than those of the AGA neonates with hypoglycemia, in the post-hoc test ( $P = 0.03$ ).

### DISCUSSION

In this study, we reported several changes in the insulin/IGF signaling pathway in cord blood derived from preterm infants. Consistent with the results of our previous study conducted with term infants, preterm SGA neonates demonstrated lower serum IGF-1 levels than preterm AGA infants. No difference was observed in the mRNA expression levels of *IRS1*, *IGF1R*, and *INSR* between the AGA and SGA preterm infants. This was similar to the observations in term infants.<sup>24</sup> In contrast, preterm SGA infants showed decreased levels of serum insulin and increased levels of *SLC2A4* mRNA, which was translated to GLUT4 glucose-transporter protein, compared to preterm AGA infants, unlike in term infants.<sup>24</sup> No statistical difference was observed in *IRS2* mRNA expression, which was increased in the full-term SGA infants, as observed in a previous study.<sup>24</sup> This is in contrast with the observations for term infants.<sup>24</sup>

The present study with preterm neonates demonstrated lower serum IGF-1 and insulin levels in SGA infants than in preterm AGA infants. The term SGA infants also exhibited lower serum IGF-1 levels.<sup>24</sup> Similar findings are reported in previous studies conducted on human infants, rats, and sheep.<sup>28–34</sup> It is well known that undernutrition induces a decrease in IGF and



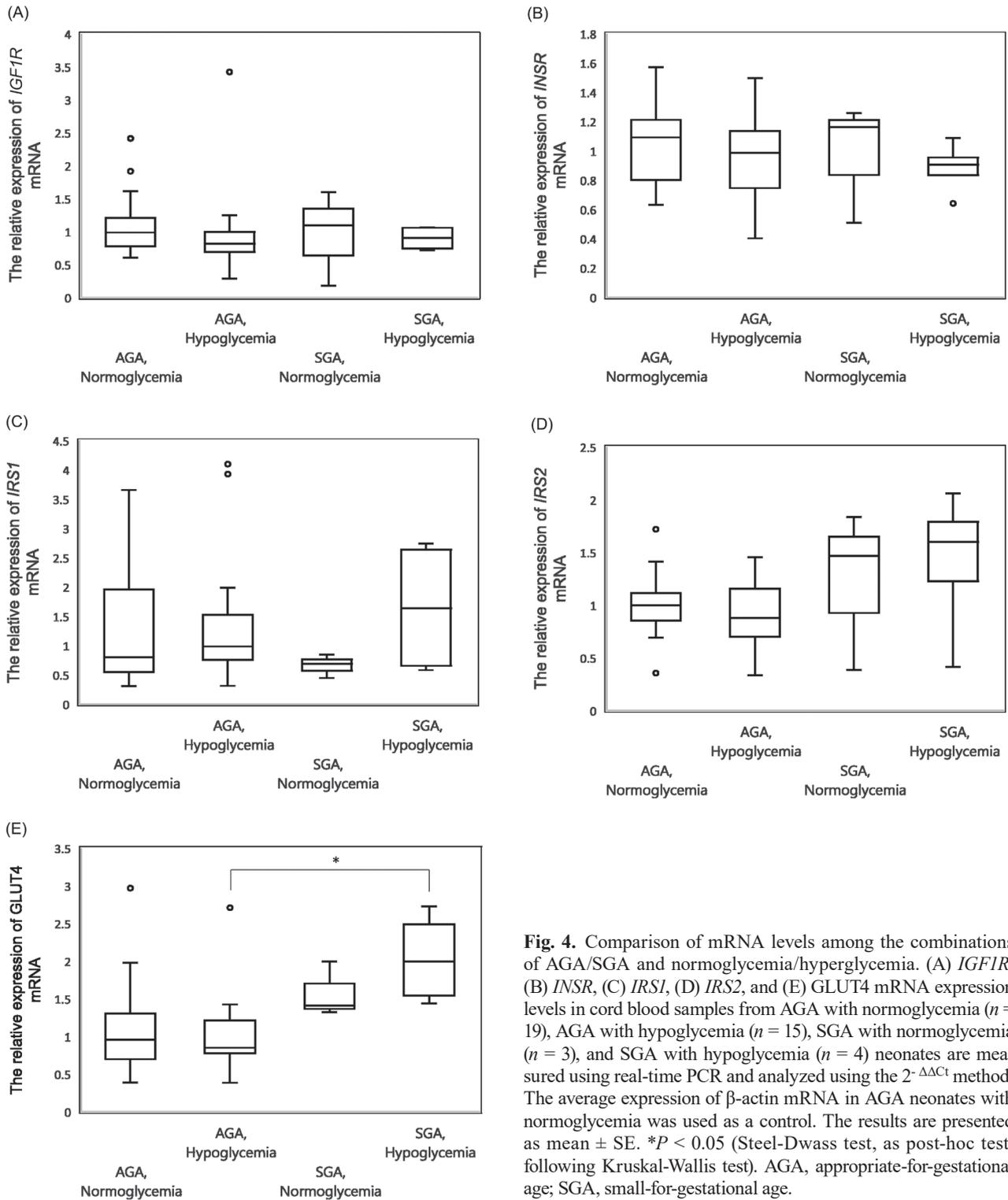
**Fig. 3.** Difference of each mRNA expression between AGA and SGA. *IGF1R*, *INSR*, *IRS1*, *IRS2*, and *GLUT4* mRNA expression levels in cord blood samples from AGA ( $n = 34$ ) and SGA ( $n = 7$ ) neonates were measured using real-time PCR and analyzed using the  $2^{-\Delta\Delta C_t}$  method. The average expression of  $\beta$ -actin mRNA in AGA neonates with normoglycemia was used as a control. The results are presented as mean  $\pm$  SE. \* $P < 0.05$  (Welch  $t$ -test). AGA, appropriate-for-gestational age; SGA, small-for-gestational age.

insulin levels and results in growth failure.<sup>1-3</sup> SGA is most commonly thought to be a malnourished condition in utero; thus, the results of this study are in accordance with previous reports.

No difference was observed in the mRNA expression of *IR* and *IGF1R*. In studies using fetal pig adipose tissue<sup>35</sup> and fetal sheep tissues (gut, placentas, and liver),<sup>32, 36</sup> no significant difference was observed between AGA and intrauterine growth restriction conditions with respect to *INSR* and *IGF1R* mRNA expression. In contrast, previous studies demonstrated that hypoglycemia in SGA neonates was caused by hyperinsulinemia and insulin sensitivity.<sup>37, 38</sup> High levels of insulin and IGF are known to decrease protein and mRNA expression of *IR* and *IGF-1R*.<sup>39</sup> Our findings suggest that the SGA prenatal neonates were not exposed to hyperinsulinemia, at least in fetal cord blood, which is consistent with the results of a previous study.<sup>24</sup> Furthermore, it is unclear whether low serum levels of IGF-1 and insulin influence *INSR* and *IGF1R* mRNA transcription. Our findings suggest that lower serum levels of IGF-1 and insulin do not change *INSR* and *IGF1R* mRNA expression levels in cord blood.

In our study, *GLUT4* mRNA expression in the cord blood was significantly higher in the preterm SGA than in the preterm AGA, and the expression in the preterm SGA with hypoglycemia was significantly higher than that in the hypoglycemic preterm AGA. However, a previous term study on term infants showed a slight increase in *GLUT4* mRNA expression, but without significance.<sup>24</sup> *GLUT4* is an important protein that facilitates the uptake of extracellular glucose into the cell. This insulin-regulated glucose transporter is primarily observed in adipose tissues and muscles.<sup>40</sup> As the SGA subjects had low serum insulin levels, the expression of *GLUT4* mRNA seemed to be increased independently of insulin. Hence, our data correlating *GLUT4* mRNA levels to preterm SGA might be related to a different factor. Although the underlying mechanism is uncertain, some reports have shown a relationship between nutritional status and *GLUT4* mRNA.<sup>35, 41-43</sup> For instance, Gondret et al. reported that term neonatal adipose tissue increases *GLUT4* mRNA expression with spontaneous intrauterine growth restriction in pigs during the last trimester of pregnancy.<sup>35</sup> Gavete et al. reported that *GLUT4* and *GLUT1* expression in 10-day-old SGA rat pup muscles were not influenced; however, fractionation studies have shown improvements in insulin-stimulated *GLUT4* translocation to the plasma membrane along with a high insulin sensitivity and an increase in tyrosine-phosphorylated insulin receptor and *IRS1*.<sup>44</sup> Toyoshima et al. also reported that protein deprivation upregulates the *IR/IRS/PI3K/mTORC1* pathway in the muscles and liver of rats, leading to increased insulin sensitivity and improved glucose uptake.<sup>45</sup> These studies suggest that undernutrition induces improved glucose uptake and increased insulin sensitivity in the early phase. However, the reason for the increased *GLUT4* mRNA expression being observed only in preterm SGA remains unclear, and further studies are needed to support this hypothesis.

We show that *GLUT4* and *IRS2* mRNA expression differed between our previous term study and this preterm study, whereas they showed a similar trend. Moreover, preterm and SGA infants have similar glucose metabolism characteristics (e.g., hypoglycemia).<sup>46</sup> Meanwhile, differences between preterm and SGA neonates with malnutrition have also been reported. For instance, it has been reported that intrauterine growth failure due to malnutrition reduces  $\beta$  cell mass.<sup>47</sup> Further, the system for glucose metabolism, including the glucose transporter, gradually matures according to gestational age,<sup>48</sup> although there is no study on mRNA expression of *IRS2* and *GLUT4* in preterm cord blood. Taken together, these findings suggest that “preterm



**Fig. 4.** Comparison of mRNA levels among the combinations of AGA/SGA and normoglycemia/hyperglycemia. (A) *IGF1R*, (B) *INSR*, (C) *IRS1*, (D) *IRS2*, and (E) *GLUT4* mRNA expression levels in cord blood samples from AGA with normoglycemia ( $n = 19$ ), AGA with hypoglycemia ( $n = 15$ ), SGA with normoglycemia ( $n = 3$ ), and SGA with hypoglycemia ( $n = 4$ ) neonates are measured using real-time PCR and analyzed using the  $2^{-\Delta\Delta Ct}$  method. The average expression of  $\beta$ -actin mRNA in AGA neonates with normoglycemia was used as a control. The results are presented as mean  $\pm$  SE. \* $P < 0.05$  (Steel-Dwass test, as post-hoc test, following Kruskal-Wallis test). AGA, appropriate-for-gestational age; SGA, small-for-gestational age.

SGA” indicates the combination of prematurity and malnutrition, and our results and previous findings point to the possibility of different pathological conditions between preterm SGA and term SGA.

Our study has some limitations that need to be

addressed. First, the sample size was small. Out of the 7 SGA patients, there were only 3 patients without and 4 with hypoglycemia. Moreover, we analyzed the mRNA expression at different time points in the previous term study and could not analyze the expression of *SLC2A2*

mRNA due to technical limitations. In addition, we used neonatal umbilical cord blood cells that consist of several components (monocytes, lymphocytes, and red cells). We analyzed the amount of mRNA expression but did not assess the effects of cell type on this expression. Accordingly, there is also the limitation of generalization of the results. Therefore, we need to increase the sample size to assess the exact mRNA expression using preterm and full-term samples at the same time, and acquire adequate tissue for accurate assessment of insulin/IGF signaling in prenatal neonates.

In summary, we investigated insulin/IGF signaling in the cord blood of preterm infants and observed that SGA preterm conditions altered the components associated with the insulin/IGF signaling pathway, but not in the same way as observed in term SGA. Our data as well as previous studies, suggest that this may be due to prematurity, adaptation to malnutrition, or both. Our study suggests the possibility of differences between the pathological conditions in preterm and term SGA. Further studies are needed to fully understand the regulation of the insulin/IGF signaling pathway during fetal development.

*Acknowledgments:* We express our deepest appreciation to the participants of this study and their families. We also thank the healthcare professionals of the Center for Maternal, Fetal, and Neonatal Medicine, Tottori University Hospital, and members of the Division of Reproductive-Perinatal Medicine and Gynecologic Oncology, Faculty of Medicine, Tottori University, Tottori, Japan. We would like to thank Editage (www.editage.com) for English language editing.

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

*The authors declare no conflicts of interest.*

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