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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.[1–3] 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.[1] IRS1 and IRS2 are considered critical intracellular proteins for insulin/IGF signaling.[1–3] Numerous molecular studies have revealed that abnormalities in the insulin/IGF signaling pathway induce insulin resistance and type 2 diabetes.[4–6] For example, increased release of free fatty acids, glycerol, hormones (*e.g.*, leptin, adiponectin, and endothelin-1),[3, 4, 6, 7] and pro-inflammatory cytokines (*e.g.*, tumor necrosis factor α, interleukin-1β, and interleukin-6) from adipose tissue in obesity, affects IRS kinase and induces abnormal signaling, resulting in insulin resistance.[8–10] 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,[11] which is occasionally associated with hyperinsulinemia, postnatal insulin resistance, and growth failure in adulthood.[12–18] In addition, preterm infants also have been shown to demonstrate abnormal growth and glucose metabolism analogous to term SGA infants.[19–21] 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.[22] In very early studies, insulin/IGF signaling was studied using human circulating blood cells.[23] Accordingly, we have

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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

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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.[24] 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 10th 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 25th and 75th 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 childbirth.[25] 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.[24] 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.[24] 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.[24] 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 β-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 β-actin expression, respectively. TaqMan Gene Expression Assay® (Hs00178563_ml) was used for *IRS1*. We calculated relative mRNA expression levels using the $2^{-\Delta\Delta Ct}$ method. The quality of samples was assessed using RNA integrity number (RIN).[26] 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),[27] 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 ± 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

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Table 1. Characteristics of preterm neonates

	Total $n = 41$	AGA $n = 34$	SGA $n = 7$	*P*-value
Maternal				
Age (years)	30 (29 to 34)	30.5 (29 to 34)	30 (29.5 to 35)	0.44
BMI (kg/cm^2)	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 (*n*, %)	17 (41)	17 (50)	0 (0)	**< 0.05**
Neonatal				
Male (*n*, %)	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)	**< 0.01**
Birth weight SDS	–0.66 (–1.23 to –0.06)	–0.48 (–0.69 to 0.01)	–1.64 (-2.39 to –1.46)	**< 0.01**
Birth length (cm)	43.5 (40 to 45.5)	44 (42 to 45.5)	38 (35 to 39.5)	**< 0.01**
Birth length SDS	–0.89 (-1.23 to –0.14)	–0.56 (–1.09 to 0.08)	–2.54 (-2.99 to –2.41)	**< 0.01**
Singleton (*n*, %)	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 (*n*, %)	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)	**< 0.05**
IGF-1 (ng/mL)	42.7 (25.4 to 56.7)	47.2 (29.85 to 59.25)	27.6 (16 to 34.5)	**< 0.05**
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 *IGF1R*		Correlation with *INSR*		Correlation with *IRS1*		Correlation with *IRS2*		Correlation with GLUT4	
	r_s	*P*	r_s	*P*	r_s	*P*	r_s	*P*	r_s	*P*
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	**< 0.05**	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 *IGF1R*		Correlation with *INSR*		Correlation with *IRS1*		Correlation with *IRS2*		Correlation with GLUT4	
r_s	*P*	r_s	*P*	r_s	*P*	r_s	*P*	r_s	*P*
–0.20	0.22	–0.15	0.35	0.06	0.73	–0.26	0.10	–0.44	**< 0.01**

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

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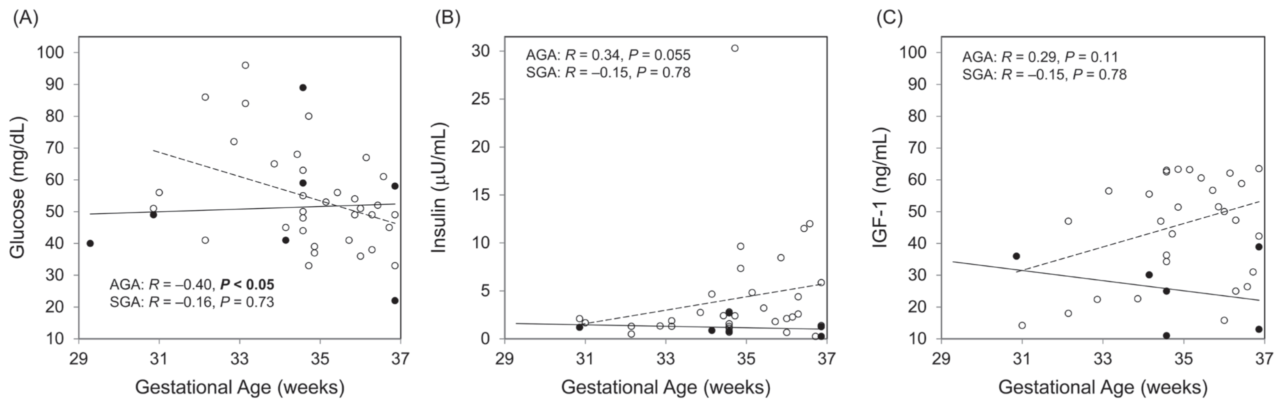

Fig. 1. Spearman correlation between gestational age and (A) glucose, (B) insulin, or (C) IGF-1. White circles and the dashed line denote AGA; black circles and the solid line denote SGA. Significant correlations ($P < 0.05$) are marked in bold. AGA, appropriate-for-gestational age; IGF-1, insulin-like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

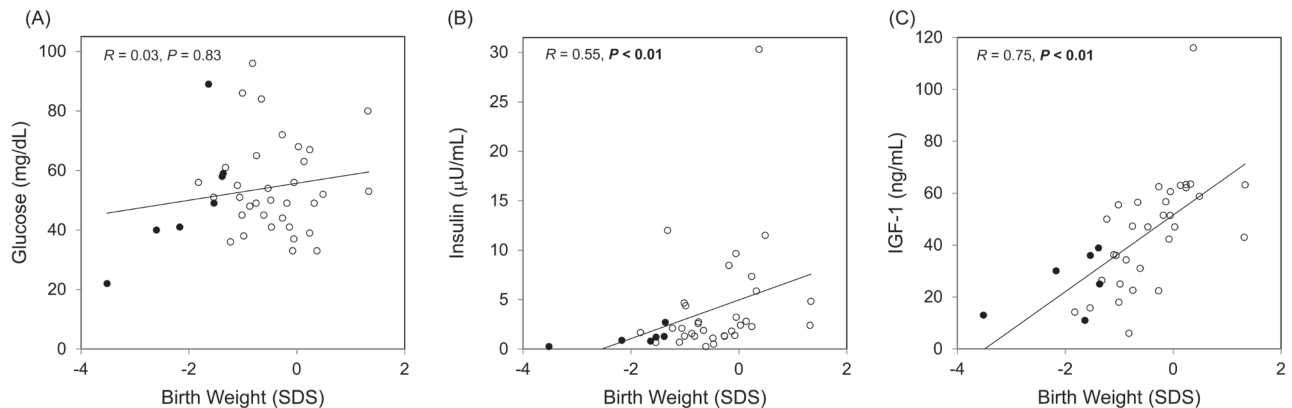

Fig. 2. Spearman correlation between birth weight SDS and (A) glucose, (B) insulin, or (C) IGF-1. White circles and the dashed line denote AGA; black circles and the solid line denote SGA. Significant correlations ($P < 0.05$) are marked in bold. AGA, appropriate-for-gestational age; IGF-1, insulin-like 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), *IRS1* mRNA in the SGA showed a negative correlation with gestational age. The mRNA expression of other genes did not correlate with gestational age.

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

		AGA		SGA		
	Total	Normoglycemia	Hypoglycemia	Normoglycemia	Hypoglycemia	*P*-value
N (*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)	**< 0.01**
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)	**< 0.05**
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.[24] 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.[24] No statistical difference was observed in *IRS2* mRNA expression, which was increased in the full-term SGA infants, as observed in a previous study.[24] This is in contrast with the observations for term infants.[24]

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.[24] Similar findings are reported in previous studies conducted on human infants, rats, and sheep.[28–34] It is well known that undernutrition induces a decrease in IGF and

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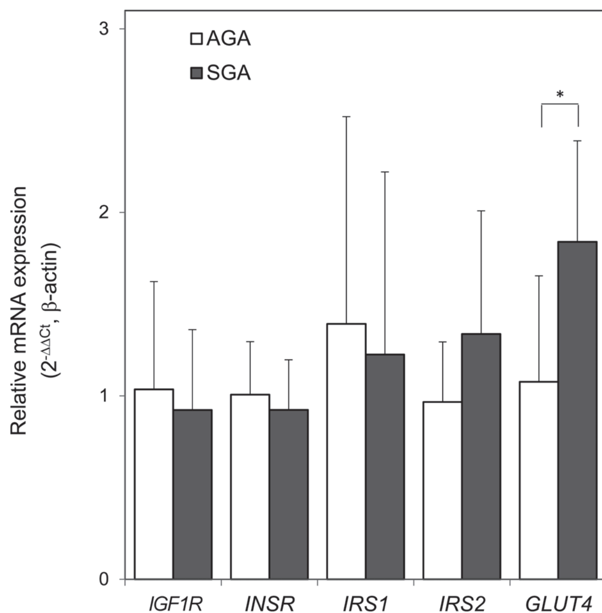

Fig. 3. Difference of each mRNA expression between AGA and SGA. *IGF1R*, *INSR*, *IRS1*, *IRS2*, and GLUT4 mRNA expression levels in cord blood samples from AGA (n = 34) and SGA (n = 7) neonates were measured using real-time PCR and analyzed using the $2^{-\Delta\Delta Ct}$ method. The average expression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

insulin levels and results in growth failure.[1–3] 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[35] and fetal sheep tissues (gut, placentas, and liver),[32, 36] 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.[37, 38] High levels of insulin and IGF are known to decrease protein and mRNA expression of IR and IGF-1R.[39] 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.[24] 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.[24] 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.[40] 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.[35, 41–43] 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.[35] 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.[44] 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.[45] 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).[46] 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 β cell mass.[47] Further, the system for glucose metabolism, including the glucose transporter, gradually matures according to gestational age,[48] although there is no study on mRNA expression of *IRS2* and GLUT4 in preterm cord blood. Taken together, these findings suggest that "preterm

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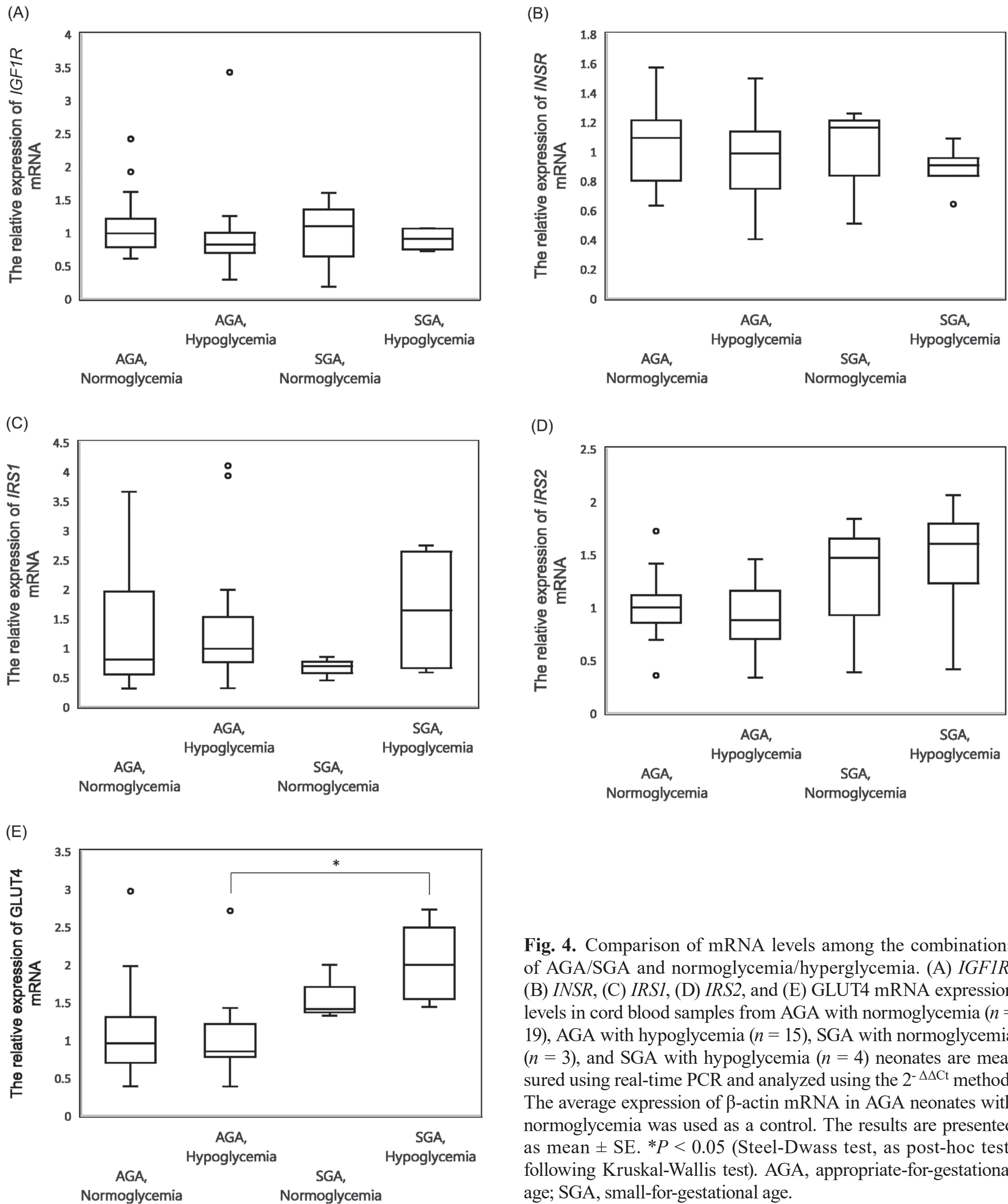

Fig. 4. Comparison of mRNA levels among the combinations of AGA/SGA and normoglycemia/hyperglycemia. (A) *IGF1R*, (B) *INSR*, (C) *IRS1*, (D) *IRS2*, and (E) GLUT4 mRNA expression levels in cord blood samples from AGA with normoglycemia (n = 19), AGA with hypoglycemia (n = 15), SGA with normoglycemia (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 β-actin mRNA in AGA neonates with normoglycemia was used as a control. The results are presented as mean ± 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*

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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.

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The authors declare no conflicts of interest.

REFERENCES

1 Boucher J, Kleinridders A, Kahn CR. Insulin receptor signaling in normal and insulin-resistant states. Cold Spring Harb Perspect Biol. 2014;6(1):a009191. DOI: 10.1101/cshperspect.a009191, PMID: 24384568
2 Adams TE, Epa VC, Garrett TPJ, Ward CW. Structure and function of the type 1 insulin-like growth factor receptor. Cell Mol Life Sci. 2000;57:1050-93. DOI: 10.1007/PL00000744, PMID: 10961344
3 Clemmons DR. Metabolic actions of insulin-like growth factor-I in normal physiology and diabetes. Endocrinol Metab Clin North Am. 2012;41:425-43, vii-viii. DOI: 10.1016/j.ecl.2012.04.017, PMID: 22682639
4 Brady MJ. IRS2 takes center stage in the development of type 2 diabetes. J Clin Invest. 2004;114:886-8. DOI: 10.1172/JCI23108, PMID: 15467824
5 Saini V. Molecular mechanisms of insulin resistance in type 2 diabetes mellitus. World J Diabetes. 2010;1:68-75. DOI: 10.4239/wjd.v1.i3.68, PMID: 21537430
6 Kahn BB, Flier JS. Obesity and insulin resistance. J Clin Invest. 2000;106:473-81. DOI: 10.1172/JCI10842, PMID: 10953022
7 Wilkes JJ, Hevener A, Olefsky J. Chronic endothelin-1 treatment leads to insulin resistance in vivo. Diabetes. 2003;52:1904-9. DOI: 10.2337/diabetes.52.8.1904, PMID: 12882904
8 Swaroop J, Naidu JN, Rajarajeswari D. Association of TNF-α with insulin resistance in type 2 diabetes mellitus. Indian J Med Res. 2012;135:127-30. DOI: 10.4103/0971-5916.93435, PMID: 22382194
9 Jager J, Grémeaux T, Cormont M, Le Marchand-Brustel Y, Tanti JF. Interleukin-1beta-induced insulin resistance in adipocytes through down-regulation of insulin receptor substrate-1 expression. Endocrinology. 2007;148:241-51. DOI: 10.1210/en.2006-0692, PMID: 17038556
10 Harder-Lauridsen NM, Krogh-Madsen R, Holst JJ, Plomgaard P, Leick L, Pedersen BK, et al. Effect of IL-6 on the insulin sensitivity in patients with type 2 diabetes. Am J Physiol Endocrinol Metab. 2014;306:E769-78. DOI: 10.1152/ajpendo.00571.2013, PMID: 24473436
11 Mejri A, Dorval VG, Nuyt AM, Carceller A. Hypoglycemia in term newborns with a birth weight below the 10th percentile. Paediatr Child Health. 2010;15:271-5. DOI: 10.1093/pch/15.5.271, PMID: 21532790
12 Putzker S, Pozza SB-D, Kugler K, Schwarz HP, Bonfig W. Insulin resistance in young adults born small for gestational age (SGA). J Pediatr Endocrinol Metab. 2014;27:253-9. DOI: 10.1515/jpem-2013-0292, PMID: 24152890
13 Veening MA, van Weissenbruch MM, Delemarre-van de Waal HA. Glucose tolerance, insulin sensitivity, and insulin secretion in children born small for gestational age. J Clin Endocrinol Metab. 2002;87:4657-61. DOI: 10.1210/jc.2001-011940, PMID: 12364453
14 Jaquet D, Gaboriau A, Czernichow P, Levy-Marchal C. Insulin resistance early in adulthood in subjects born with intrauterine growth retardation. J Clin Endocrinol Metab. 2000;85:1401-6. DOI: 10.1210/jcem.85.4.6544, PMID: 10770173
15 Veening MA, van Weissenbruch MM, Heine RJ, Delemarre-van de Waal HA. Beta-cell capacity and insulin sensitivity in prepubertal children born small for gestational age: influence of body size during childhood. Diabetes. 2003;52:1756-60. DOI: 10.2337/diabetes.52.7.1756, PMID: 12829643
16 Collins JE, Leonard JV, Teale D, Marks V, Williams DM, Kennedy CR, et al. Hyperinsulinaemic hypoglycaemia in small for dates babies. Arch Dis Child. 1990;65:1118-20. DOI: 10.1136/adc.65.10.1118, PMID: 2248501
17 Barker DJP, Hales CN, Fall CHD, Osmond C, Phipps K, Clark PMS. Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. Diabetologia. 1993;36:62-7. DOI: 10.1007/BF00399095, PMID: 8436255
18 Geremia C, Cianfarani S. Insulin sensitivity in children born small for gestational age (SGA). Rev Diabet Stud. 2004;1:58-65. DOI: 10.1900/RDS.2004.1.58, PMID: 17491667
19 Hofman PL, Regan F, Jackson WE, Jefferies C, Knight DB, Robinson EM, et al. Premature birth and later insulin resistance. N Engl J Med. 2004;351:2179-86. DOI: 10.1056/NEJMoa042275, PMID: 15548778

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20 Rotteveel J, van Weissenbruch MM, Twisk JWR, Delemarre-Van de Waal HA. Infant and childhood growth patterns, insulin sensitivity, and blood pressure in prematurely born young adults. Pediatrics. 2008;122:313-21. DOI: 10.1542/peds.2007-2012, PMID: 18676549
21 Hofman PL, Regan F, Cutfield WS. Prematurity--another example of perinatal metabolic programming? Horm Res. 2006;66:33-9. DOI: 10.1159/000093230, PMID: 16685134
22 Eshet R, Klinger B, Silbergeld A, Laron Z. Modulation of insulin like growth factor I (IGF-I) binding sites on erythrocytes by IGF-I treatment in patients with Laron syndrome (LS). Regul Pept. 1993;48:233-9. DOI: 10.1016/0167-0115(93)90352-9, PMID: 8265812
23 Beck-Nielsen H, Pedersen O, Kragballe K, Sorensen NS. The monocyte as a model for the study of insulin receptors in man. Diabetologia. 1977;13:563-9. DOI: 10.1007/BF01236308, PMID: 590649
24 Fujimoto M, Sonoyama YK, Fukushima K, Imamoto A, Miyahara F, Miyahara N, et al. Increased IRS2 mRNA expression in SGA neonates: PCR analysis of insulin/IGF signaling in cord blood. J Endocr Soc. 2017;1:1408-16. DOI: 10.1210/js.2017-00294, PMID: 29264464
25 Itabashi K, Miura F, Uehara R, Nakamura Y. New Japanese neonatal anthropometric charts for gestational age at birth. Pediatr Int. 2014;56:702-8. DOI: 10.1111/ped.12331, PMID: 24617834
26 Fleige S, Walf V, Huch S, Prgomet C, Sehm J, Pfaffl MW. Comparison of relative mRNA quantification models and the impact of RNA integrity in quantitative real-time RT-PCR. Biotechnol Lett. 2006;28:1601-13. DOI: 10.1007/s10529-006-9127-2, PMID: 16900335
27 Kanda Y. Investigation of the freely available easy-to-use software ‘EZR’ for medical statistics. Bone Marrow Transplant. 2013;48:452-8. DOI: 10.1038/bmt.2012.244, PMID: 23208313
28 Bazaes RA, Salazar TE, Pittaluga E, Peña V, Alegría A, Iñiguez G, et al. Glucose and lipid metabolism in small for gestational age infants at 48 hours of age. Pediatrics. 2003;111:804-9. DOI: 10.1542/peds.111.4.804, PMID: 12671116
29 Duan C, Liu M, Xu H, Tang W, Liu J, Hou L, et al. Decreased expression of GLUT4 in male CG-IUGR rats may play a vital role in their increased susceptibility to diabetes mellitus in adulthood. Acta Biochim Biophys Sin (Shanghai). 2016;48:872-82. DOI: 10.1093/abbs/gmw088, PMID: 27614316
30 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:E1716-25. DOI: 10.1152/ajpendo.00459.2007, PMID: 17895285
31 Chiesa C, Osborn JF, Haass C, Natale F, Spinelli M, Scapillati E, et al. Ghrelin, leptin, IGF-1, IGFBP-3, and insulin concentrations at birth: is there a relationship with fetal growth and neonatal anthropometry? Clin Chem. 2008;54:550-8. DOI: 10.1373/clinchem.2007.095299, PMID: 18202160
32 Duffield JA, Vuocolo T, Tellam R, Yuen BS, Muhlhausler BS, McMillen IC. Placental restriction of fetal growth decreases IGF1 and leptin mRNA expression in the perirenal adipose tissue of late gestation fetal sheep. Am J Physiol Regul Integr Comp Physiol. 2008;294:R1413-9. DOI: 10.1152/ajpregu.00787.2007, PMID: 18272661
33 Thorn SR, Regnault TRH, Brown LD, Rozance PJ, Keng J, Roper M, et al. Intrauterine growth restriction increases fetal hepatic gluconeogenic capacity and reduces messenger ribonucleic acid translation initiation and nutrient sensing in fetal liver and skeletal muscle. Endocrinology. 2009;150:3021-30. DOI: 10.1210/en.2008-1789, PMID: 19342452
34 Kyriakakou M, Malamitsi-Puchner A, Mastorakos G, Boutsikou T, Hassiakos D, Papassotiriou I, et al. The role of IGF-1 and ghrelin in the compensation of intrauterine growth restriction. Reprod Sci. 2009;16:1193-200. DOI: 10.1177/1933719109344629, PMID: 19770497
35 Gondret F, Père MC, Tacher S, Daré S, Trefeu C, Le Huërou-Luron I, et al. Spontaneous intra-uterine growth restriction modulates the endocrine status and the developmental expression of genes in porcine fetal and neonatal adipose tissue. Gen Comp Endocrinol. 2013;194:208-16. DOI: 10.1016/j.ygcen.2013.09.018, PMID: 24095810
36 Shaikh S, Bloomfield FH, Bauer MK, Phua HH, Gilmour RS, Harding JE. Amniotic IGF-I supplementation of growth-restricted fetal sheep alters IGF-I and IGF receptor type 1 mRNA and protein levels in placental and fetal tissues. J Endocrinol. 2005;186:145-55. DOI: 10.1677/joe.1.06113, PMID: 16002544
37 Wiswell TE, Cornish JD, Northam RS. Neonatal polycythemia: frequency of clinical manifestations and other associated findings. Pediatrics. 1986;78:26-30. PMID: 3725498
38 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;68:269-73. DOI: 10.1136/adc.68.3_Spec_No.269, PMID: 8466261
39 Taniguchi CM, Emanuelli B, Kahn CR. Critical nodes in signalling pathways: insights into insulin action. Nat Rev Mol Cell Biol. 2006;7:85-96. DOI: 10.1038/nrm1837, PMID: 16493415
40 Huang S, Czech MP. The GLUT4 glucose transporter. Cell Metab. 2007;5:237-52. DOI: 10.1016/j.cmet.2007.03.006, PMID: 17403369
41 Lie S, Morrison JL, Williams-Wyss O, Suter CM, Humphreys DT, Ozanne SE, et al. Periconceptional undernutrition programs changes in insulin-signaling molecules and microRNAs in skeletal muscle in singleton and twin fetal sheep. Biol Reprod. 2014;90:5. DOI: 10.1095/biolreprod.113.109751, PMID: 24258211
42 Thamotharan M, Shin BC, Suddirikku DT, Thamotharan S, Garg M, Devaskar SU. GLUT4 expression and subcellular localization in the intrauterine growth-restricted adult rat female offspring. Am J Physiol Endocrinol Metab. 2005;288:E935-47. DOI: 10.1152/ajpendo.00342.2004, PMID: 15625086
43 Muhlhausler BS, Duffield JA, Ozanne SE, Pilgrim C, Turner N, Morrison JL, et al. The transition from fetal growth restriction to accelerated postnatal growth: a potential role for insulin signalling in skeletal muscle. J Physiol. 2009;587:4199-211. DOI: 10.1113/jphysiol.2009.173161, PMID: 19622603
44 Gavete ML, Martín MA, Alvarez C, Escrivá F. Maternal food restriction enhances insulin-induced GLUT-4 translocation and insulin signaling pathway in skeletal muscle from suckling rats. Endocrinology. 2005;146:3368-78. DOI: 10.1210/en.2004-1658, PMID: 15905322

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45 Toyoshima Y, Tokita R, Ohne Y, Hakuno F, Noguchi T, Minami S, et al. Dietary protein deprivation upregulates insulin signaling and inhibits gluconeogenesis in rat liver. J Mol Endocrinol. 2010;45:329-40. DOI: 10.1677/JME-10-0102, PMID: 20801894
46 Sharma A, Davis A, Shekhawat PS. Hypoglycemia in the preterm neonate: etiopathogenesis, diagnosis, management and long-term outcomes. Transl Pediatr. 2017;6:335-48. PMID: 29184814
47 Hay WW Jr. Placental-fetal glucose exchange and fetal glucose metabolism. Trans Am Clin Climatol Assoc. 2006;117:321-39. PMID: 18528484
48 Mitanchez D. Glucose regulation in preterm newborn infants. Horm Res. 2007;68:265-71. DOI: 10.1159/000104174, PMID: 17587854

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