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## Cord Blood Irisin Levels are Positively Correlated with Birth Weight in Newborn Infants

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### Abstract

**Background**—Irisin is a novel myokine, secreted from skeletal muscle after exercise. Irisin mediates exercise-related energy expenditure by turning white adipose tissue (WAT) into brown adipose tissue (BAT). Thus, irisin is considered as a potential biomarker for obesity and metabolic syndrome. Infants born small for gestational age (SGA) have increased risk for metabolic syndrome. However, the physiologic role of irisin in neonates remains to be studied.

**Objective**—To evaluate the association of umbilical cord blood irisin levels with gestational age and birth weight categories in neonates.

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The authors do not have any conflicts of interests

Author contribution:

KJ designed study, performed experiments and analysis and drafted the manuscript. HC oversaw umbilical cord blood sample collection and processing and clinical data entry, contributed to study design and edited the manuscript. KH contributed in statistical analysis of data. FD and AF performed laboratory measurements. CM conceptualized the study and supervised all steps including laboratory measurement, analysis of data, and reviewed the final manuscript.

**Methods**—A cross-sectional study of 341 newborns, from 26 to 41 weeks' gestation. We collected umbilical cord blood and analyzed plasma for irisin by ELISA.

**Results**—Plasma irisin levels were positively correlated with gestational age ( $r=0.21$ ,  $p<0.001$ ), and birth weight Z-score ( $r=0.18$ ,  $p<0.001$ ). SGA infants had significantly lower irisin (median [interquartile range] 55.38 [46.56 - 65.72] ng/mL) compared to appropriate for gestational age infants (64.41 [53.87 - 76.76] ng/mL) and large for gestational age infants (68.70 [54.78 - 79.09] ng/mL,  $p<0.01$ ). The association between SGA and lower irisin remained significant in multivariate analysis independent of gestational age, maternal age, maternal BMI, and gestational diabetes ( $p=0.03$ ). In singleton infants, irisin was also significantly negatively associated with maternal preeclampsia ( $p=0.01$ ).

**Conclusions**—Our results support the notion that irisin may have a physiologic role in neonates. We speculate that decreased levels of irisin in SGA infants may contribute to the development of catch up growth and metabolic syndrome later in life.

### Keywords

cord blood; irisin; birth weight; newborn

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### Introduction

Irisin, a novel regulator of metabolic function, is known for its role in exercise-induced energy expenditure and 'browning' of white adipose tissue [1]. Irisin is produced by cleavage of its precursor, fibronectin type III domain containing 5 (FNDC 5), in skeletal muscle after exercise-induced stimulation by peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ) coactivator **1-alpha (PGC-1 $\alpha$ )** [1, 2]. Circulating irisin acts on white adipose tissue, an energy-saving organ, and results in its 'conversion' to brown adipose tissue (BAT), which is an energy-producing organ. This is mediated by thermogenesis via the uncoupling protein 1 (UCP1) pathway. Animal studies showed that irisin improved glucose tolerance in obese, pre-diabetic mice [1]. In clinical settings, patients with type 2 diabetes mellitus have lower levels of irisin compared to healthy controls [3, 4], and irisin's precursor, FNDC5, is decreased in patients with obesity [5]. However, circulating irisin is reported to be paradoxically higher in adults with metabolic syndrome [6], which suggests that states of irisin resistance or tolerance may exist [7, 8].

Newborn infants have predominantly high content of brown adipose tissue (BAT) for thermogenesis, and the content of brown fat decreases significantly after infancy [9]. Small for gestational age (SGA) infants have lower percentage of total body fat (% body fat) compared to appropriate for gestational age (AGA) infants [10-12], which may result in lower irisin levels. SGA and intrauterine growth restriction (IUGR) are known risk factors for metabolic syndrome in adult life [13]. However, the mechanism underlying the association between SGA and metabolic syndrome later in life is not fully understood. Thus, we examined the levels of irisin in the umbilical cord blood between different birth weight categories, and examined the association between cord blood irisin levels and birth weight Z-score and gestational age across a range of gestational ages from 26 to 41 weeks.

## METHODS

### Study population

This is a cross-sectional study of 341 neonates. Neonates included in the study were born at Brigham and Women's Hospital (BWH) from April 2010 to November 2013. Collection of umbilical cord blood was performed from all vaginal and Cesarean deliveries when the research nurse or an investigator were available, and when the cord blood was not needed for another purpose such as cord blood banking. The study was reviewed and approved by the Institutional Review Board (IRB) of Brigham and Women's Hospital (BWH). Analysis of discarded material (cord blood) and review of medical record do not require a written consent from the parents. The study adheres to the declaration of Helsinki.

We excluded newborns with major congenital anomalies such as congenital heart and renal anomalies or maternal chronic inflammatory disease, such as Crohn's disease, ulcerative colitis, systemic lupus erythematosus (SLE) or cancer.

Among the 341 infants included in analysis, 217 were full term (≥ 37 weeks), and 124 were preterm (35-36 weeks: 51, 32-34 weeks: 29, 29-31 weeks: 26, 26-28 weeks: 18). Determination of gestational age was based on best obstetrical estimate from the medical record. Newborns were categorized as small for gestational age (SGA: Birth weight <10<sup>th</sup> percentile), appropriate for gestational age (AGA: 10<sup>th</sup> percentile Birth weight 90<sup>th</sup> percentile), and large for gestational age (LGA: Birth weight >90<sup>th</sup> percentile) groups based on the intrauterine growth curves [14].

### Cord blood collection and measurement of plasma irisin

Umbilical cord blood was collected from the umbilical vein attached to the placenta at the time of delivery. They were centrifuged and the plasma was divided into 0.5 milliliter aliquots, which were stored in Eppendorf tubes at -80°C until analysis. Levels of irisin were measured by ELISA by commercial kits (EK 067-52, Phoenix Pharmaceutical, Burlingame, CA, USA) with 1:2 dilution of plasma sample (70 µL). Intra- and inter-assay variances were <4-6% and <8-10%, and the range of detectable concentration was 0.066-1024 ng/mL.

### Clinical data of newborn infants and mothers

We collected clinical information from the electronic medical records. Maternal data included age, race/ethnicity, marital status, parity, multiple gestation versus singleton, insurance, smoking status, pre-pregnancy body mass index (BMI), clinical chorioamnionitis, preeclampsia and gestational diabetes. Infant data included sex, gestational age, mode of delivery, birth weight, 1 and 5 minute Apgar scores. Birth weight Z scores were obtained using a reference by Fenton et al [14]. Diagnosis of maternal preeclampsia was made by systolic blood pressure ≥140mmHg and/or diastolic ≥90mmHg after 20 weeks of gestation, and proteinuria ≥0.3g protein in 24 hour urine or spot urine protein/creatinine ratio ≥0.3 mg/mg [15]. Diagnosis of gestational diabetes was made by glucose loading test with 50g glucose, and confirmed by subsequent diagnostic oral glucose challenge test (OGTT) with 100g glucose. Gestational diabetes was diagnosed when two or more of the following

criteria were met: the glucose level at fasting was >95 mg/dL, 1 hour glucose >180 mg/dL, 2 hour >155 mg/dL, 3 hour >140mg/dL [16].

Clinical chorioamnionitis was diagnosed with maternal fever >100.4°F, and two out of four additional findings (uterine tenderness, maternal tachycardia, fetal tachycardia, foul/purulent amniotic fluid) [17].

### Statistical Analysis

Kruskal-Wallis test was used for continuous variables for comparison between the three different birth weight categories (SGA, AGA, LGA). *Post-hoc* pairwise analyses between two groups were performed by Wilcoxon rank sum test with Bonferroni correction ( $p < 0.017$  was considered significant in post-hoc analyses). Chi square test and Fisher's exact tests were used for categorical variables. Normality of irisin was tested with Shapiro-Wilk test, and the data was transformed to logarithmic scale to obtain normality. Spearman correlation analyses were performed between continuous variables including irisin, gestational age, birth weight Z-score, maternal age and BMI. We performed multiple regression analysis with irisin as an outcome variable including those variables with  $p < 0.1$  in univariate analyses and maternal BMI, infant sex. We used SPSS 19 (Chicago, IL, USA) for statistical analysis.

## RESULTS

### Patient characteristics (Table 1)

Among 341 newborn infants, 60 (17.6%) were SGA, 257 (75.4%) were AGA, and 24 (7.0%) were LGA infants. The median gestational age and birth weight were lower in the SGA group compared to AGA and LGA groups. Median irisin level was significantly lower in SGA infants (median [interquartile range] 55.4 [46.6-65.7] ng/mL) compared to AGA (64.4 [53.9-76.7] ng/mL) and LGA (68.7 [54.8-79.1] ng/mL) groups ( $p < 0.001$ , Table 1 and Figure 1A). Gestational age and birth weight were significantly lower in SGA compared with AGA and LGA groups ( $p < 0.001$ ), and there were more infants from multiple gestation pregnancies in the SGA group compared to AGA and LGA groups ( $p < 0.001$ ). Maternal age was lower in AGA group compared to SGA and LGA groups ( $p = 0.02$ ), and there were more primigravid mothers in SGA group ( $p = 0.03$ ). There was no difference in the maternal complications related to pregnancy such as gestational diabetes, preeclampsia, and chorioamnionitis between groups.

### Correlation between gestational age, birth weight, and circulating irisin levels

Full term infants had higher levels of irisin (67.16 [56.51-83.92] ng/mL) compared to preterm infants (56.9 [48.93-68.32] ng/mL,  $p = 0.001$ ) and there was a significant positive correlation between plasma irisin levels and gestational age ( $r = 0.21$ ,  $p < 0.001$ , Table 2).

In correlation analysis, we found a significant positive linear correlation between cord blood irisin levels and birth weight Z-score ( $r = 0.18$ ,  $p < 0.001$ , Table 2 and Fig. 1B). Maternal age was negatively correlated with irisin level ( $r = -0.12$ ,  $p = 0.02$ , Table 2). Maternal BMI was not associated with cord blood irisin ( $r = 0.02$ ,  $p = 0.79$ , Table 2).

### Multivariate regression analysis for factors associated with cord blood irisin levels (Table 3)

In univariate linear regression analysis, cord blood levels of irisin were associated with gestational age, birth weight Z-score, maternal age and race/ethnicity, gravidity, delivery by C-section, multiple gestation, and maternal preeclampsia with  $p < 0.1$ . Multivariate regression analysis was performed in stepwise models as shown in Table 3. Variables listed above and clinically important demographic variables such as maternal BMI and infant sex were included in the final model. Birth weight Z-score was independently associated with cord blood levels of irisin ( $\beta = 0.05$ ,  $p = 0.008$ , Table 3A). Gestational age was not independently associated with irisin in multivariate regression analysis ( $\beta = 0.005$ ,  $p = 0.43$ ). In a model which included SGA, and LGA, with AGA as the reference group instead of using birth weight Z-score to assess the association of SGA and LGA statuses and irisin, the result showed that SGA was an independent factor associated with lower irisin ( $\beta = -0.10$ ,  $p = 0.03$ , Table 3B). LGA status was not associated with cord blood irisin levels ( $\beta = 0.09$ ,  $p = 0.25$ , Table 3B).

### Sub-group analysis of singleton infants (n=236, Table 4)

When we excluded all infants who were products of multiple gestations, SGA remained a significant independent predictor of lower cord blood irisin levels ( $\beta = -0.14$ ,  $p = 0.02$ ). Maternal preeclampsia ( $\beta = -0.23$ ,  $p = 0.01$ ) and age ( $\beta = -0.008$ ,  $p = 0.04$ ) were negatively associated with circulating irisin levels in the cord blood (Table 4).

### Discussion

Our cross-sectional study shows that cord blood irisin levels are positively correlated with birth weight Z-scores and gestational age, and the levels are decreased in SGA compared to AGA and LGA infants. To our knowledge, this is the first report of irisin cord blood levels in relation to the birth weight Z-scores and gestational age. The positive correlation between birth weight Z-score and irisin remained significant after controlling for potential confounding variables such as gestational age, maternal age, maternal BMI, and infant sex. Our results also reveal that singleton infants of mothers with preeclampsia had lower cord blood irisin levels compared to infants of mothers without preeclampsia.

A recent small study of irisin levels in umbilical cord arterial blood revealed positive correlation between irisin and fetal weight, and a statistically significant lower levels of irisin in growth restricted infants [18]. The authors did not find a difference between cord blood venous levels and maternal blood levels of irisin in the same study population. Our findings are in agreement with this prior study although our study samples were a mixture of arterial and venous blood. No LGA infants were included in the prior study. In contrast, our study included LGA infants as well as SGA infants for comparison. While it is plausible that the differences in skeletal muscle content between SGA, AGA and LGA infants is the main underlying reason for the observed differences, our results revealed that there is no significant difference in cord blood irisin levels between LGA and AGA/SGA population. We speculate that cord blood irisin levels are more relevant to the fetal growth restricted state, and may not play an important role in overgrowth, although this may be due to the small number of LGA infants in our sample (n=24).

Another study reported that irisin is detectable in cord blood and correlates with maternal circulating levels [19]. Although we did not examine maternal blood irisin levels, we included important maternal factors such as gestational diabetes, preeclampsia, BMI, and age as potential confounders. Compared to a prior study of circulating levels of irisin in healthy adults (Park et al, n=107) [6], the levels we detected in cord blood in neonates are significantly lower (162.2 [133.5–206.9] ng/mL vs 63.25 [53.99 - 74.60] ng/mL). This needs to be confirmed and its etiology should be explored further, but it may be due to lower body fat mass and/or muscle mass in newborns compared to adults or different regulation of irisin during fetal life.

Garces et al recently reported that irisin's precursor, FNDC 5, is detectable by immunohistochemistry in placental tissues from normal pregnancies including the decidua, cytotrophoblast, and syncytiotrophoblast cells [20]. They also reported that circulating irisin levels in pregnant women with preeclampsia were lower compared to women with healthy pregnancies in the third trimester. In this previous study, umbilical cord blood irisin levels were not measured. In our current study, we found that circulating cord blood irisin levels were negatively associated with preeclampsia among singleton neonates. The current knowledge of the pathophysiology of preeclampsia includes inadequate trophoblast invasion and abnormal placental development, which lead to hypoperfusion and ischemia to the placenta [21]. Thus, our results suggest that low irisin levels may be related to abnormal placentation and/or placental insufficiency, in the setting of preeclampsia. However, our study does not prove a causal relationship between irisin and preeclampsia, and the underlying molecular mechanisms need to be investigated in future studies.

Circulating irisin levels in pediatric populations have not been widely studied. A recently published study in children reported that plasma levels of irisin are higher in females compared to males, are positively correlated with BMI, and are negatively correlated with insulin resistance as assessed by the homeostasis model assessment-estimated insulin resistance (HOMA-IR) [22]. We did not find an association between infant sex and irisin levels in our newborn population. Differences in fat content and distribution of adipose tissue between females and males in older children may account for the discrepancy in sexual dimorphism in irisin levels between these two age groups. In adults, there is a controversial relationship between irisin and BMI. A positive correlation was found among healthy subjects in three studies, but a negative correlation was found in patients with metabolic diseases in two studies [7]. Since BMI does not have important clinical implications in the newborn period, we used birth weight Z-score in our study, and found that irisin is positively correlated with birth weight Z-score independent of gestational age.

Previous studies in adults showed significantly lower circulating irisin levels in patients with type 2 DM compared to healthy controls [3, 4, 7]. Interventional studies have documented an increase in irisin after acute exercise, which may prove to be a therapeutic target in glucose homeostasis [8]. Recent reports of irisin in relation to gestational diabetes mellitus (GDM) have shown controversial results between maternal circulating irisin levels and GDM [19, 23, 24]. In our study, offspring of mothers with GDM did not have significantly different circulating irisin levels compared to offspring of mothers without GDM (data not shown). Our findings are in agreement with the reports by Yuksel et al and Piya et al, who also found



no difference in circulating irisin levels in cord blood of infants of mothers with GDM and mothers without GDM [19, 23]. In the study of Yuskel et al, in contrast to cord blood, maternal serum irisin was lower in the GDM group compared to the control group. One study measuring CSF irisin levels of mothers at the time of delivery revealed that irisin is significantly elevated in CSF in women with GDM compared to non-GDM [19].

Irisin is also found in human breast milk, but its role in growth and weight gain of neonates and infants is unknown [25]. Animal studies in sheep revealed that browning of white adipose tissue was stimulated by irisin via activation of the Mitogen Activated Protein Kinases (MAP kinase) p38 and Erk signaling and that this pathway was disrupted by maternal nutritional restriction in late pregnancy [26]. In a study of nutritionally deprived newborn sheep, there was decreased expression of brown adipose tissue related genes such as uncoupling protein (UCP)-1 and beta 3 adrenergic receptor ( $\beta$ 3ADR), whereas the expression of white adipose tissue related genes were not affected [9]. This suggests that brown adipose tissue may be more significantly affected compared to white adipose tissue in fetal growth restriction.

Differences in white and brown adipose tissue mass between SGA and AGA newborn human infants have not been studied. Although direct measurement of brown fat by 18-FDG PET is not feasible in the newborn population, further studies measuring markers of brown fat, such as uncoupling protein (UCP)-1, could reveal an association between circulating irisin levels and the amount of brown fat in newborns of different birth weight categories more accurately. SGA infants are also known to have lower skeletal muscle mass compared to AGA infants [10] and this may directly affect the levels of irisin through decreased production. Future studies including direct measurement of lean body mass or muscle enzymes such as creatine kinase, aldolase and lactate dehydrogenase will be needed to evaluate this association. With current study design, we cannot conclude whether the difference in circulating irisin levels in SGA and LGA groups is related more to differences in fat content or skeletal muscle content. Low irisin levels in SGA infants may also be related to lower energy expenditure, which could potentially lead to fast weight gain and catch-up growth later in life. Since rapid catch-up growth is a known risk factor for obesity and metabolic syndrome [27], this association may reveal a potentially new pathophysiologic explanation regarding the development of obesity and metabolic syndrome in this vulnerable population. Further prospective studies could reveal a potential association more precisely.

The limitations of our study include a relatively high-risk study population, with a high number of preterm and SGA infants in addition to a high proportion of multiple pregnancies, based in a tertiary medical center, which may limit the generalizability of our findings. Our study population had high proportion of babies born via C-section reflecting high-risk deliveries. However, additional subgroup analysis of babies born via spontaneous vaginal delivery (n=83), also showed the same significant difference between SGA, AGA, and LGA infants ( $p=0.049$ ) as is shown in the analysis of total population, and multiple linear regression analysis also showed statistically significant association between SGA status and lower level of cord blood irisin ( $\beta=-0.30$ ,  $p=0.02$ ). Although, there have been debates in the literature regarding the optimal methodology for measuring circulating irisin levels, we used

the validated ELISA kit and method described in our previous studies as mentioned above [2, 6].

Finally, we did not collect maternal blood for this study, thus, the effect of maternal plasma level on cord blood irisin cannot be evaluated. As previously mentioned, our cord samples are mixed, therefore it is not possible to determine the origin of irisin in the samples. Our study raises a possibility that irisin may mediate the relationship between SGA status at birth and later metabolic syndrome however a direct causal relationship cannot be confirmed by our study. This needs to be addressed in a longitudinal study of irisin levels and postnatal weight gain as well as future onset obesity and metabolic syndrome.

In summary, we found that cord blood irisin levels are positively correlated with birth weight Z-score and are significantly lower in SGA infants compared to AGA, and LGA infants. Cord blood irisin levels are negatively associated with maternal preeclampsia among singleton infants. Decreased irisin levels in SGA infants may be related to low content of brown adipose tissue and irisin may link the association between fetal growth restriction and the higher incidence of obesity and metabolic syndrome in adult life.

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## Abbreviations

<b>PPAR<math>\gamma</math></b>	peroxisome proliferator-activated receptor gamma
<b>PGC-1<math>\alpha</math></b>	PPAR $\gamma$ -coactivator 1 alpha
<b>FNDC5</b>	fibronectin type III domain containing 5
<b>UCP1</b>	uncoupling protein 1
<b>BMI</b>	body mass index
<b>BAT</b>	brown adipose tissue
<b>GDM</b>	gestational diabetes mellitus
<b>HOMA-IR</b>	homestasis model assessment-estimated insulin resistance

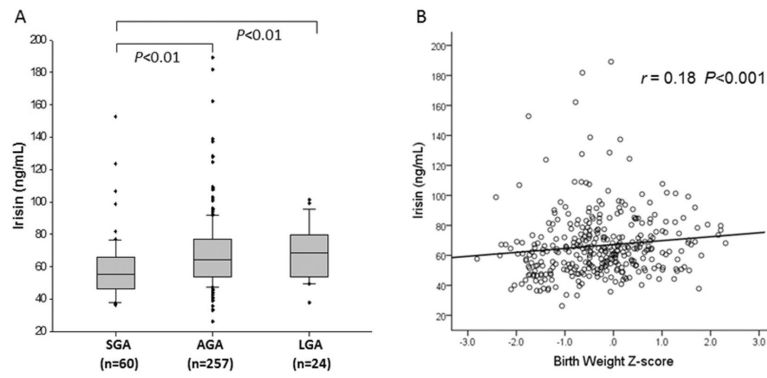
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**Fig. 1.**

A. Cord blood irisin levels in relation to birth weight categories. Kruskal-Wallis test reveals a significant difference between the levels of irisin in SGA compared to AGA and LGA infants. Bars represent median, boxes represent 25<sup>th</sup> and 75<sup>th</sup> percentile (interquartile range), and whiskers represent 95% confidence interval. Circles represent extreme values. B. Correlation analysis (Spearman correlation coefficient) between plasma irisin and birth weight Z-score.

Table 1

## Patients Characteristics

	All (n=341)	SGA (n=60)	AGA (n=257)	LGA (n=24)	P
<b>Newborn Characteristics</b>					
Irisin (ng/mL) <sup>a</sup>	63.3 (52.0, 74.6)	55.4 (46.6, 65.7)	64.4 (53.9, 76.7)	68.7 (54.8, 79.1)	<0.001
Gestational Age (weeks) <sup>b</sup>	38.1 (35.4, 39.1)	36.9 (35.1, 38.4)	38.0 (35.4, 39.1)	39.4 (39.0, 39.9)	<0.001
Birth Weight (Kg) <sup>b</sup>	2.9 (2.2, 3.5)	2.3 (1.9, 2.6)	3.1 (2.5, 3.5)	4.3 (4.2, 4.5)	<0.001
Birth Weight Z-score <sup>b</sup>	-0.3 (-1.0, 0.3)	-1.6 (-1.8, -1.5)	-0.2 (-0.6, 0.3)	1.6 (1.4, 1.8)	<0.001
C-section, n (%)	258 (75.7)	50 (83.3)	190 (73.9)	18 (75.0)	0.31
Multiple Gestation, n (%)	105 (30.8)	35 (58.3)	69 (26.9)	1 (4.2)	<0.001
Female Sex, n (%)	173 (50.7)	36 (60.0)	125 (48.6)	12 (50.0)	0.28
1 min Apgar	8 (8-9)	8 (7-8)	8 (8-9)	8 (8-9)	0.21
5 min Apgar	9 (9-9)	9 (8-9)	9 (9-9)	9 (9-9)	0.08
<b>Maternal Characteristics</b>					
Maternal Age (years)	33 (30, 37)	34.0 (31.0, 38.0)	32.0 (30.0, 36.0)	34.5 (32.0, 37.0)	<b>0.02</b>
Maternal Race <sup>c</sup>	(n=337)	(n=59)	(n=254)	(n=24)	
White, n (%)	206 (60.4)	36 (60.0)	154 (59.9)	16 (66.7)	0.84
Non-White, n (%)	131 (38.4)	23 (38.3)	100 (38.9)	8 (33.3)	
Smoking (yes), n (%) <sup>c</sup>	(n=329) 7 (2.1)	(n=56) 1 (1.7)	(n=256) 6 (2.3)	(n=23) 0 (0)	1.00
Primigravida, n (%)	123 (36.1)	26 (43.3)	94 (36.6)	3 (12.5)	<b>0.03</b>
Maternal BMI (Kg/m <sup>2</sup> ) <sup>c</sup>	(n=324) 24.0 (21.6, 28.3)	(n=59) 24.0 (21.4, 27.7)	(n=243) 24.0 (21.4, 28.3)	(n=22) 27.0 (22.7, 33.4)	0.05
Preeclampsia, n (%)	31 (9.1)	8 (13.3)	23 (9.0)	0 (0)	0.15
Chorioamnionitis, n (%)	10 (2.9)	0 (0)	10 (3.9)	0 (0)	0.33
Gestational Diabetes, n (%) <sup>c</sup>	(n=299) 27 (9.0)	(n=56) 3 (5.4)	(n=222) 21 (9.5)	(n=21) 3 (14.3)	0.42
PROM>18 hrs, n (%)	14 (6.2)	3 (5.0)	17 (6.6)	1 (4.2)	0.92

Abbreviations: SGA, small for gestational age; AGA, appropriate for gestational age; LGA, large for gestational age; BMI, body mass index; PROM, premature rupture of membrane

Note: Variables are presented with median (interquartile range), or n (%),  $p < 0.05$  in bold, Kruskal-Wallis test (continuous variables), Chi-square or Fisher's exact test (categorical variables)

Post-hoc pairwise analysis between two groups by Wilcoxon rank-sum test with Bonferroni correction ( $P < 0.017$  was considered significant)

<sup>a</sup>Significant difference between SGA group and AGA/LGA groups

<sup>b</sup>Significant difference between all three groups (SGA vs AGA, AGA vs LGA, and SGA vs LGA)

<sup>c</sup>Data with missing values, n is presented

**Table 2**

Correlation analysis of irisin, gestational age, birth weight Z-score, and maternal BMI

	<b>Irisin (ng/mL)</b>	<b>Gestational age (weeks)</b>	<b>Birth weight Z-score</b>	<b>Maternal Age (years)</b>	<b>Maternal BMI (Kg/m<sup>2</sup>)</b>
Gestational Age (weeks)	$r = 0.21$ $p < 0.001$	1			
Birth weight Z-score	$r = 0.18$ $p = 0.001$	$r = 0.25$ $p < 0.001$	1		
Maternal Age (year)	$r = -0.12$ $p = 0.02$	$r = -0.13$ $p = 0.01$	$r = 0.06$ $p = 0.31$	1	
Maternal BMI (Kg/m <sup>2</sup> )	$r = 0.02$ $p = 0.79$	$r = 0.06$ $p = 0.25$	$r = 0.14$ $p = 0.01$	$r = 0.02$ $p = 0.72$	1

Abbreviations: BMI, body mass index

Spearman correlation coefficient

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**Table 3**

**A. Multivariate Linear Regression Analysis (Outcome: log(Irisin))**

Variables	Model 1 <sup>a</sup>		Model 2		Model 3		Model 4	
	$\beta$ (95% C.I.)	<i>P</i>	$\beta$ (95% C.I.)	<i>P</i>	$\beta$ (95% C.I.)	<i>P</i>	$\beta$ (95% C.I.)	<i>P</i>
Birth Weight Z-score	0.05 (0.02 – 0.08)	<b>0.003</b>	0.04 (0.10 – 0.07)	<b>0.01</b>	0.05 (0.01 – 0.08)	<b>0.007</b>	0.05 (0.01 – 0.09)	<b>0.008</b>
Gestational Age (weeks)			0.01 (0.001 – 0.02)	<b>0.03</b>	0.005 (–0.005 – 0.01)	0.31	0.005 (–0.01 – 0.02)	0.43
Multiple Gestation					–0.04 (–0.11 – 0.04)	0.35	–0.01 (–0.10 – 0.08)	0.79
Maternal Age (yr)					–0.01 (–0.01 – 0.001)	0.08	–0.01 (–0.01 – 0.001)	0.09
Maternal Race (non-White)					0.04 (–0.03 – 0.10)	0.26	0.04 (–0.04 – 0.11)	0.30
Maternal BMI (Kg/m <sup>2</sup> )					–0.001 (–0.01 – 0.01)	0.59	–0.0003 (–0.01 – 0.01)	0.93
Gestational Diabetes							0.01 (–0.11 – 0.13)	0.85
Maternal Preeclampsia							–0.07 (–0.19 – 0.05)	0.25
Mode of Delivery (C-section)							–0.05 (–0.14 – 0.04)	0.26
Primigravida							–0.04 (–0.11 – 0.03)	0.22
Infant Sex (Female)							0.01 (–0.06 – 0.08)	0.72

**B. Multivariate Linear Regression Analysis (Outcome: log (Irisin))**

Variables	Model 1 <sup>a</sup>		Model 2		Model 3		Model 4	
	$\beta$ (95% C.I.)	<i>P</i>	$\beta$ (95% C.I.)	<i>P</i>	$\beta$ (95% C.I.)	<i>P</i>	$\beta$ (95% C.I.)	<i>P</i>
SGA (Ref: AGA)	–0.15 (–0.23 – –0.07)	<b>&lt;0.001</b>	–0.15 (–0.23 – –0.07)	<b>&lt;0.001</b>	–0.12 (–0.20 – –0.04)	<b>0.005</b>	–0.10 (–0.19 – –0.01)	<b>0.03</b>
LGA (Ref: AGA)	0.04 (–0.09 – 0.17)	0.52	0.02 (–0.12 – 0.15)	0.82	0.06 (–0.08 – 0.19)	0.40	0.09 (–0.06 – 0.24)	0.25
Gestational Age (weeks)			0.01 (0.003 – 0.02)	<b>0.01</b>	0.006 (–0.003 – 0.02)	0.21	0.006 (–0.01 – 0.02)	0.39
Multiple Gestation					–0.05 (–0.12 – 0.30)	0.24	–0.03 (–0.11 – 0.06)	0.56
Maternal Age (yr)					–0.004 (–0.10 – 0.002)	0.17	–0.005 (–0.01 – 0.002)	0.16
Maternal Race (non-White)					–0.03 (–0.03 – 0.10)	0.34	0.03 (–0.04 – 0.10)	0.41
Maternal BMI (Kg/m <sup>2</sup> )					–0.001 (–0.01 – 0.004)	0.60	–0.0004 (–0.01 – 0.01)	0.90
Gestational Diabetes							0.02 (–0.10 – 0.14)	0.74
Maternal Preeclampsia							–0.08 (–0.20 – 0.04)	0.18
Mode of Delivery (C-section)							–0.04 (–0.13 – 0.05)	0.35



Variables	Model 1 <sup>a</sup>		Model 2		Model 3		Model 4	
	$\beta$ (95% C.I.)	<i>P</i>	$\beta$ (95% C.I.)	<i>P</i>	$\beta$ (95% C.I.)	<i>P</i>	$\beta$ (95% C.I.)	<i>P</i>
Primigravida							-0.05 (-0.12 – 0.02)	0.20
Infant Sex (Female)							0.01 (-0.05 – 0.08)	0.70

Abbreviations: BMI, body mass index; SGA, small for gestational age; AGA, appropriate for gestational age; LGA, large for gestational age

<sup>a</sup>Model 1: unadjusted univariate regression

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**Table 4**

Multiple Regression Analysis for a subgroup of singleton Infants(n=236)

Variables	$\beta$ (95% C.I.)	SE	P
SGA (Ref: AGA)	-0.14 (-0.26 – -0.03)	0.06	<b>0.02</b>
LGA (Ref: AGA)	-0.04 (-0.11 – 0.19)	0.08	0.58
Gestational Age (weeks)	0.006 (-0.01 – 0.02)	0.008	0.44
Maternal Age (year)	-0.008 (-0.02 – -0.001)	0.004	<b>0.04</b>
Maternal Race (non-White)	0.07 (-0.01 – 0.15)	0.04	0.11
Maternal BMI (Kg/m <sup>2</sup> )	0.005 (-0.002 – 0.01)	0.003	0.17
Gestational Diabetes	0.02 (-0.13 – 0.17)	0.08	0.82
Maternal Preeclampsia	-0.23 (-0.40 – -0.06)	0.09	<b>0.01</b>
Mode of Delivery (C-section)	-0.03 (-0.12 – 0.06)	0.05	0.48
Primigravida	-0.06 (-0.15 – 0.02)	0.04	0.14
Infant Sex (Female)	-0.02 (-0.09 – 0.05)	0.04	0.70

Abbreviations: SGA, small for gestational age; AGA, appropriate for gestational age; LGA, large for gestational age; LGA, large for gestational age; BMI, body mass index

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