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Research Article

Serum irisin levels are correlated with insulin resistance in women with gestational diabetes mellitus

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Abstract

Objectives: The aim of this study was to determine the serum irisin level in pregnant women with gestational diabetes mellitus (GDM) and those with a normal glucose tolerance test (NGT).

Methods: Forty-three pregnant women who underwent a 50-g glucose challenge test (GCT) at 24-28 gestational weeks were included the study. If the serum glucose level result of the 50-g GCT was >140 mg/dL, a 100-g oral glucose tolerance test (OGTT) was applied. Diagnosis of GDM was confirmed if any 2 of the blood glucose test results were above the following levels: fasting serum glucose ≥92 mg/dL, 1-hour glycemia ≥180 mg/dL, or 2-hour glycemia ≥153 mg/dL. Patients with a result below this value were accepted as NGT. Nine pregnant patients with 50-g GCT results below 140 mg/dL and 5 pregnant women with a history of chronic hypertension were excluded. The remaining 29 pregnant women underwent a 100-g OGTT and were divided into 2 groups according to their results: 15 had GDM and 14 had a NGT. Maternal serum irisin was measured using an enzyme immunoassay method. The correlation between biochemical and demographic parameters and the serum irisin level in all participants were analyzed.

Results: There were no significant difference between the GDM and NGT groups in terms of the maternal serum irisin level (8.71±1.34 µg/mL vs 8.17±1.01 µg/mL; p>0.32). The levels of fasting insulin, fasting glucose, and the 2-hour glucose level, as well as the homeostatic model assessment of insulin resistance (HOMA-IR) results of patients with GDM were higher than those of the NGT group. The maternal serum irisin level of the GDM subjects were positively and significantly correlated with the HOMA-IR value (r:0.65; p<0.05), fasting insulin level (r:0.44; p<0.05), and body mass index (BMI) (r:0.81; p<0.05). A negative but significant correlation between the serum irisin level and diastolic blood pressure of the GDM subjects was also seen. Insignificant associations were found between the HOMA-IR score, fasting insulin level, BMI, and the serum irisin level of the NGT subjects.

Conclusion: The maternal serum irisin level may regulate the HOMA-IR value, fasting insulin level, and diastolic blood pressure in women with GDM.

Keywords: Body mass index, gestational diabetes mellitus, homeostatic model assessment of insulin resistance, irisin, insulin

Gestational diabetes mellitus (GDM) is a metabolic disease that negatively affects the health of the mother and the fetus. It is the most frequent complication of pregnancy. For this reason, follow-up and treatment is required with a multidisciplinary approach. If the necessary attention is not given to the diagnosis and follow-up, GDM may negatively affect the future health of both the mother and the fetus [1]. The children of mothers with GDM are at risk for obesity, diabetes mellitus, and other endocrine diseases [2].

Comparable to the role of adipose tissue as an endocrine gland, skeletal muscle fibers produce and secrete myokines. Many myokines are secreted by muscle cells; in addition to irisin, they include fibroblast growth factor 21, decorin, and myonectin [3]. Other muscle-derived factors detected are brain-derived neurotrophic factor and osteocrin [4]. The presence of these myokines supports the endocrine role of skeletal muscle. Through these proteins, muscle cells can communicate with other tissues, through both autocrine and paracrine

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activity. For all of these reasons, it is thought that skeletal muscle is an endocrine organ.

Irisin is a newly described membrane protein that is synthesized by skeletal muscle myocytes and secreted into systemic circulation [5, 6]. Its precursor is fibronectin type III domain-containing protein 5 (FNDC5). Both production and secretion of irisin are maintained by peroxisome proliferator-activated receptor gamma coactivator 1-alpha in the circulation [5, 7]. Exercise is also one of the most important factors that regulates irisin synthesis and release. Circulating irisin is involved in glucose and insulin homeostasis by increasing the amount of brown fat tissue [5].

There are several studies reporting that production and secretion of serum irisin have changed in some metabolic disorders, including polycystic ovary syndrome, type 2 diabetes mellitus, and GDM [8, 9]. Decreased serum irisin levels have been reported in women with GDM compared with healthy controls [10]. On the other hand, several studies have reported that serum irisin levels of women with GDM were similar to those of non-GDM subjects. That is, the data on irisin levels in patients with GDM are not clear. A recent meta-analysis conducted by Zhao et al. [10] reported that significantly reduced serum irisin levels were detected in GDM patients compared with healthy pregnant subjects. In the present study, the aim was to investigate the circulating irisin level of women with GDM and those with a 50-g glucose challenge test (GCT) value above 140 mg/dL but a 100-g oral glucose tolerance test (OGTT) that was normal. Possible associations between the circulating irisin level and biochemical and demographic parameters were also analyzed.

Materials and Methods

Diagnosis of gestational diabetes mellitus

Pregnant women with the laboratory criteria meeting the definition of GDM according to well-established rules were enrolled the study [11]. Forty-three pregnant women were screened with a 50-g GCT at 24-28 weeks of gestation. If the serum glucose level was greater than 140 mg/dL on the 50-g GCT, a 100-g OGTT was applied. Diagnosis of GDM was confirmed if 2 of the blood glucose test results were above the following levels: fasting serum glucose ≥92 mg/dL and/or 1-hour glycemia ≥180 mg/dL, and/or 2-hour glycemia ≥153 mg/dL. The blood samples used in this study were obtained from frozen sera used in a previous study evaluating breast milk irisin levels in GDM [1]. Venous blood samples were taken for the measurement of serum irisin levels and other hormonal markers. The mean gestational week at birth was 37.1±0.2. Nine pregnant patients with 50-g GCT results below 140 mg/dL and 5 pregnant women with a history of chronic hypertension were excluded. The remaining 29 pregnant women underwent a 100-g OGTT and were divided into 2 groups according to the results: 15 had GDM (range: 26-32 years of age) and 14 had a normal glucose tolerance test (NGT) (range: 25-31 years of age). A possible correlation between biochemical and demographic parameters and the circulating irisin level in GDM and NGT subjects was analyzed. Participants with a multiple-pregnancy history, pre-existing glucose intolerance or DM, current cigarette use, or acute or chronic inflammation were also excluded. The body mass index (BMI) was calculated using the following formula: weight [kg]/square meter height [m^2] at the time of delivery. For each participant, the height and weight were evaluated using standard methods. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) measurements were made on the left arm with the patient in a sitting position. Measurement was performed shortly before delivery and when the patient was fasting. If the measurements were higher than expected, a second evaluation was done and the results were averaged. The present study conformed to the principles outlined in the Declaration of Helsinki. It was conducted using frozen blood samples from a previous study with prior ethical approval for their collection and use. All of the patients provided verbal, informed consent prior to participation in the study protocol.

Glucose and insulin analysis

The serum glucose levels were measured in an autoanalyzer using the hexokinase/G6PD method (Architect c16000; Abbott Laboratories, Lake Bluff, IL, USA). The fasting serum insulin levels were also measured in an autoanalyzer using an electrochemiluminescence immunoassay (Architect c8000; Abbott Laboratories, Lake Bluff, IL, USA). The homeostatic model assessment for insulin resistance [HOMA-IR] formula was used to calculate responsiveness [12].

Irisin analysis

Frozen blood samples were thawed and centrifuged at 3000 x g for 10 minutes before the analyses. Maternal serum irisin levels were measured using a human enzyme immunoassay (Hangzhou Eastbiopharm Co. Ltd., Hangzhou, China). The minimum detection limit for irisin was 0.05 µg/mL. The intra and inter assay coefficient of variation was less than 10% and 12%, respectively. The method developed and previously described by Aydin et al. [1] was used for the assay validation of the samples. A ChroMate P4300 microplate reader (Awareness Technology Instruments, Inc., Palm City, FL, USA) was used to analyze the circulating irisin.

Statistical analysis

SPSS for Windows, Version 15.0 software (SPSS Inc., Chicago, IL, USA) was used to perform the statistical analysis. Normality of the data was analyzed with the Kolmogorov-Smirnov Z test. A non-parametric paired t-test was used for comparisons between the GDM and NGT groups. Associations between the maternal serum irisin, biochemical, hormonal, and demographic parameters were analyzed using the Pearson correlation method. The data were presented as mean±SD. A p value <0.05 was accepted as statistically significant.

Results

Table 1 illustrates the serum irisin level and other parameters in the GDM and NGT subjects. No significant difference was detected between the GDM and NGT groups in terms of the mean serum irisin level (8.71±1.34 µg/mL vs 8.17±1.01 µg/mL; p>0.32). The fasting serum insulin level, fasting serum glucose level, 2-hour serum glucose concentration, and HOMA-IR value were found to be increased in the patients with GDM in comparison with the NGT group. Pearson correlation analysis revealed that the maternal serum irisin concentration of women with GDM was positively and significantly associated with HOMA-IR (r:0.65; p<0.05) (Table 2). Similarly, the maternal serum irisin level was positively associated with the fasting insulin concentration of the GDM subjects (r:0.44; p<0.05). A positive and significant correlation was found between the BMI value and the circulating irisin concentration in the GDM subjects (r:0.81; p<0.05). A negative but significant correlation was also detected between the serum irisin level and DBP (r:0.63; p<0.05). The maternal serum irisin concentration of the NGT group was not associated with the BMI value. Insignificant associations were found between the HOMA-IR and the fasting insulin level in the NGT subjects. Neither a positive nor a negative association was found between the irisin concentration and other biochemical and demographic parameters in the NGT subjects.

Discussion

Since irisin is able to stimulate white adipose tissue thermogenesis, it may play an important role in energy homeostasis during normal pregnancy [8]. It contributes to the regulation of energy production by providing the conversion between white and brown adipose tissue [5]. It has been reported that irisin is able to increase mitochondrial respiration and induce thermogenesis in human brown adipocytes [4]. Treatment with irisin increases the browning of subcutaneous adipose tissue. Many clinical studies have investigated the irisin concentrations in women with GDM during pregnancy or just after giving birth.

A detailed review of the recent literature revealed that many investigations have found a wide range of serum irisin levels in GDM subjects. Most authors have reported that circulating irisin levels were lower in women with GDM compared with pregnant women with normal glucose levels [8, 13, 14]. Likewise, decreased levels of serum irisin have been reported in patients with type 2 diabetes mellitus [8]. In the present study, the circulating irisin level of women with GDM was clearly

Table 1. Biochemical and demographic parameters in women with GDM and NGT

	GDM (n=15)	NGT (n=14)	p
Age (years)	29.12±0.13	28.23±7.12	>0.14
Irisin (µg/mL)	8.71±1.34	8.17±1.01	>0.32
HOMA-IR	5.11±1.10	3.32±0.13	<0.01
Fasting insulin (mU/mL)	16.41±4.46	5.52±5.13	<0.002
Fasting glucose (mg/dL)	98.63±5.56	86.56±0.23	<0.001
2-hour glucose (mg/dL)	159.12±1.19	113.12±4.10	<0.001
DBP (mmHg)	70.15±4.12	68.21±1.34	>0.27
SBP (mmHg)	108.12±6.13	110.12±6.44	>0.40
BMI (kg/m^2)	28.16±4.51	27.94±1.43	>0.13
Gestational age at birth (weeks)	36.36±1.33	37.15±3.20	>0.61

BMI: Body mass index; DBP: Diastolic blood pressure; GDM: Gestational diabetes mellitus, HOMA-IR: Homeostatic model assessment insulin resistance; NGT: Normal glucose tolerance test; SBP: Systolic blood pressure.

Table 2. Pearson correlation coefficient (r) between maternal serum irisin level and other parameters in GDM and NGT groups

	GDM group maternal serum irisin level		NGT group maternal serum irisin level	
Independent variables	r	p	r	p
BMI	0.81	<0.05	0.67	>0.05
HOMA-IR	0.65	<0.05	0.43	>0.05
Fasting insulin	0.44	<0.05	0.10	>0.05
DBP	-0.63	<0.05	-0.32	>0.05
Age	0.12	>0.05	0.30	>0.05

BMI: Body mass index; DBP: Diastolic blood pressure; GDM; Gestational diabetes mellitus; HOMA-IR; Homeostatic model assessment insulin resistance; NGT: Normal glucose tolerance test.

similar to the results of pregnant women with NGT. Our results were not consistent with most studies investigating serum irisin levels in GDM [8, 13, 14]. This inconsistency may be the result of multiple possible factors. When the relevant studies were examined, it was found that the parity and manner of birth of the patients participating in the GDM studies were different [8, 13, 14]. The BMI of the patients with GDM was also very different in many studies. Although the irisin level is significantly affected by exercise, most studies have not examined whether or not the patients are physically active [5]. Another possible cause of this difference may be the time the blood samples were taken. The irisin measurement was performed in almost all of the GDM studies at the 24-28th gestational week [8]. In the present study, the blood samples used for the measurement of the serum irisin level and other hormonal markers were taken near just after given birth. This may be the most important reason why the irisin level was similar in both groups. Other possible causes of disagreement in the irisin results between our study and others include the fact that in the other studies, blood samples were taken only once for irisin measurement, so the inter-trimester change is unknown. Also, it was not reported whether insulin treatment was administered to patients diagnosed with GDM. Furthermore, the irisin measurement methods used were different in many studies.

When the literature was evaluated to examine the possible relationship between the serum irisin and glucose levels, it was found that there was not much agreement between the studies [8, 13, 14]. In the present study we did not find a clear relationship between the serum glucose and irisin levels in the GDM subjects. Unlike glucose, a correlation between irisin and insulin levels was a common finding in many studies [7, 8, 13]. It has also been reported that irisin has a critical role in insulin homeostasis in diabetic patients [7]. Similarly, we found a positive association between the serum irisin level, HOMA-IR, and fasting insulin level in GDM subjects.

Muscle cell-derived irisin may mediate energy homeostasis during pregnancy. Hence, in the present study, we focused on the possible association among the maternal serum irisin level, fasting insulin level, HOMA-IR, systolic and diastolic blood pressure, and BMI. We found a negative association between the circulating irisin level and diastolic blood pressure. We also demonstrated that the maternal serum irisin level was positively associated with the HOMA-IR and fasting insulin level of GDM subjects. Consistent with our results, Mizgier et al [15] found that skeletal muscle cells produce different types of myokines capable of modulating insulin secretion. Moreover, studies have also reported a relationship between boosted muscle oxidative capacity and insulin sensitivity in response to endurance [16, 17].

In addition, in the present study, the serum irisin level was associated positively with the BMI of women with GDM. There was no correlation between hormonal and demographic parameters and the serum irisin level in the NGT subjects. In accordance with our work, a negative association was reported between the circulating irisin level and the systolic and diastolic blood pressure in preeclamptic women [18]. Shoukry et al. [19] showed that the circulating irisin level was associated with the BMI, HOMA-IR, and the fasting insulin level in type 2 diabetes mellitus. Moreover, a recent meta-analysis reported that the serum irisin level was positively associated with an insulin resistance index in patients with a normal serum glucose level [20]. The same meta-analysis also reported that there was a positive association between the circulating irisin level and insulin resistance in subjects with a fasting blood glucose of >6.1 mmol/L. Our research did not reveal any correlation between the serum irisin level and the fasting blood glucose level in GDM and NGT subjects.

In the present study, irisin, a newly described myokine, was measured in the serum of women with GDM and healthy pregnant women without GDM just after given birth. The irisin concentration was associated positively with the HOMA-IR, fasting insulin level, and the BMI of GDM subjects. A negative correlation was found between the serum irisin level and the DBP of GDM subjects. Since the main function of maternal irisin has not yet been specified, further clinical studies are needed to clarify its role in the energy homeostasis of women with GDM.

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Conflict of interest: There is no conflict of interest.

Ethics Committee Approval: This study was conducted on frozen blood samples from a previous study, so no additional ethics committee approval was required.

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