



Contents lists available at ScienceDirect

Taiwanese Journal of Obstetrics & Gynecology

journal homepage: www.tjog-online.com

Original Article

Could serum levels of irisin be used in gestational diabetes predicting?

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ARTICLE INFO

Article history:

Accepted 26 January 2019

Keywords:

Irisin
 Gestational diabetes
 Glucose intolerance
 Hyperinsulinemia
 Maternal insulin resistance

ABSTRACT

Objective: Gestational diabetes mellitus (GDM) is a metabolic disorder during pregnancy leading to acute and chronic complications in both mother and newborn. The pathogenesis of GDM has not been fully understood. However, since the disease shares risk factors with type 2 diabetes mellitus (T2DM), a relationship between these two disease states is plausible. The recently discovered peptide irisin has been hypothesized to be a regulator of body metabolism. However, studies ended up with controversial results. In the present study, we aimed to investigate the relationship between irisin levels and gestational diabetes mellitus and the possible benefits of the metabolic profile.

Materials and methods: We performed a cross-sectional analysis of circulating levels of irisin in 100 pregnant women similar for age and body mass index and the groups included 50 gestational diabetic patients and 50 healthy pregnant volunteers. Serum irisin levels were measured by ELISA kit.

Results: Mean age and body mass index levels were similar in both groups. Median HbA1c, fasting blood glucose, Glucose 1 h, Glucose 2 h and fasting insulin levels were higher in with gestational diabetic patients compared to the control group. In gestational diabetic group, the median irisin level was lower than in the control group.

Conclusion: Serum irisin levels were lower in gestational diabetic patients. Further investigations are needed to explore the underlying biological effects of irisin on pregnant women.

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Introduction

Metabolic diseases are pandemic throughout the world and new strategies and biomarkers are researched and created for early diagnosis and treatment. Gestational diabetes mellitus (GDM) is a metabolic disorder during pregnancy leading to acute and chronic complications in both mother and newborn. The pathogenesis of GDM has not been fully understood. However, since the disease shares risk factors with type 2 diabetes mellitus (T2DM), a relationship between these two disease states is plausible [1]. In this context, the fundamental study of Boström and co-workers introduced the myokine irisin as an exercise-inducible secreted factor that improves glucose tolerance and increases energy expenditure in mice [2]. Several studies were held out to investigate irisin's role in glucose metabolism; however they ended up with controversial results [3,4]. Whereas irisin has beneficial effects in rodents, data in humans are insufficient so far to evaluate its metabolic effects and

association with metabolic disease. Thus, few data suggest that irisin is associated with insulin sensitivity and new-onset of T2DM [5,6]. However, no data on irisin regulation in GDM and pregnancy are available. Therefore, we aimed to investigate the relationship between irisin levels and GDM and the possible benefits of the metabolic profile.

Materials and methods

Pregnant women routinely tested for GDM with a 75 g 2-h oral glucose tolerance test (OGTT) at the gynecological out-patient clinic of the Medical University of Our University, 50 patients with GDM and 50 women with normal glucose tolerance (NGT), matched for age, gestational age and BMI, were recruited for this cross-sectional study. GDM was diagnosed if one or more plasma glucose levels were elevated during a 75 g, 2 h oral glucose tolerance test (oGTT) according to the criteria of the American Diabetes Association [6]. The following threshold plasma glucose levels were used: fasting ≥ 100 mg/dl (5.5 mmol/l), 1 h ≥ 180 mg/dl (10.0 mmol/l) and 2 h ≥ 140 mg/dl (7.8 mmol/l). Patients with multiple pregnancy, pre-existing glucose intolerance, pregnancy-induced hypertension,

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preeclampsia, acute or chronic inflammation, as well as active smokers were not included. Written informed consent was obtained from all participants before enrollment, and the protocol was approved by the local ethics committee.

Overnight fasting venous blood samples were obtained from all participants to assess irisin levels and other biochemical parameters in the second trimester (24–28th weeks of gestation) during GDM screening. All samples were stored at room temperature for at least 30 min to allow the blood to clot, followed by centrifugation (2500 rpm, 15 min, 4 °C) to separate serum. Serum specimens were aliquoted and stored at –80 °C until irisin levels were analyzed. Glucose levels were measured with the hexokinase method using a commercially available kit (Beckman AU5800; Beckman Coulter Diagnostics, Brea, CA). Insulin levels were determined using a chemiluminescent assay (AccessDxI800; Beckman Coulter, Inc., Fullerton, CA). Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated using as fasting glucose (mmol/L) \times fasting insulin (IU/mL)/22.5 [7]. Serum irisin levels were quantified using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Eastbiopharm, Hangzhou, China). The sensitivity of the assay was 23 ng/ml, and inter- and intra-assay coefficients of variation were both $\leq 10\%$. All samples were analyzed in duplicate. Assay results are expressed as ng/mL.

Statistical analysis

For discrete and continuous variables, descriptive statistics (mean, standard deviation, median, minimum value, maximum value, and percentile) were given. In addition, the homogeneity of the variances, which is one of the prerequisites of parametric tests, was checked through Levene's test. The assumption of normality was tested via the Shapiro–Wilk test. To compare the differences between the two groups, the Student's *t* test was used when the parametric test prerequisites were fulfilled, and the Mann Whitney–U test was used when such prerequisites were not fulfilled. The relationship between the two continuous variables was assessed by Pearson Correlation Coefficient and the Spearman Correlation Coefficient when the parametric test prerequisites were

not met. Linear regression analysis was used between continuous variables. The data were evaluated via SPSS 20 (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.). $p < 0.05$ and $p < 0.01$ were taken as significance levels.

Results

Table 1 summarizes the demographic characteristics and laboratory findings of study population.

Mean age ($p = 0.140$) and BMI ($p = 0.059$) levels were similar in both groups. Gravida was higher in the gestational diabetes group than control group (2.96 ± 1.59 vs 1.8 ± 0.71 , $p = 0.001$, respectively). Median HbA1c ($5.64 \pm 0.56\%$ vs $5.23 \pm 0.37\%$, $p < 0.001$, respectively), FBG (102.2 ± 10.91 mg/dl vs 83.44 ± 6.98 mg/dl, $p < 0.001$, respectively), Glucose 1 h (175.4 ± 10.34 mg/dl vs 147.36 ± 13.95 mg/dl, $p < 0.001$, respectively), Glucose 2 h (142.2 ± 9.43 mg/dl vs 116.56 ± 13.86 mg/dl, $p < 0.001$, respectively) and FI (13.28 ± 5.63 pmol/l vs 6.99 ± 2.91 , $p < 0.001$, respectively) levels were higher in with GDM patients compared to the control group. While median creatinine, CRP, TSH, ALT, AST, Homocysteine, LDL-C, TG, Total-C levels were similar between the two groups, HDL-C levels (60.76 ± 10.76 mg/l vs 74.28 ± 17.82 mg/l, $p < 0.001$, respectively) were lower compared to the control group. In GDM group, the median irisin level (39.2 ± 28.55 ng/ml vs 69.06 ± 96.62 ng/ml, $p = 0.015$, respectively) was lower than in the control group (Fig. 1).

Discussion

The present study showed that serum irisin levels were significantly lower in the patients with GDM than in the healthy pregnant women. Early diagnosis and appropriate treatment of GDM is helpful in reducing the adverse maternal and fetal outcomes and also in protecting mothers and infants from long-term complications. Due to the major effects of irisin on the metabolism, many studies have been previously conducted to investigate the association between irisin and metabolic diseases. Irisin levels have often been determined to be decreased in T2DM [6] and increased in

Table 1
Demographic characteristics and laboratory findings of study populations.

	Gestational Diabet n = 50	Control n = 50	p
Age (years)	30.28 \pm 6.99	27.52 \pm 5.82	0.140
Gravida	2.96 \pm 1.59	1.8 \pm 0.71	0.001**
BMI (kg/m ²)	26.72 \pm 1.84	25.08 \pm 3.23	0.059
Gestational age at blood sampling (week)	25.56 \pm 0.51	25.48 \pm 0.51	0.580
HbA1c (%)	5.64 \pm 0.56	5.23 \pm 0.37	0.001**
FBG (mg/dl)	102.2 \pm 10.91	83.44 \pm 6.98	0.001**
Glucose 1 h (mg/dl)	175.4 \pm 10.34	147.36 \pm 13.95	0.001**
Glucose 2 h (mg/dl)	142.2 \pm 9.43	116.56 \pm 13.86	0.001**
FI (pmol/l)	13.28 \pm 5.63	6.99 \pm 2.91	0.001**
HOMA-IR	2.51 \pm 0.83	1.84 \pm 0.58	0.001**
Creatinine (mg/dl)	0.87 \pm 0.14	0.78 \pm 0.18	0.060
CRP (mg/l)	15.71 \pm 21.62	8.45 \pm 5.39	0.110
TSH (mIU/L)	2 \pm 0.9	1.7 \pm 0.98	0.260
ALT (U/L)	24.64 \pm 53.91	10.08 \pm 3.7	0.180
AST (U/L)	25.36 \pm 23.85	17.08 \pm 4.14	0.090
Homocysteine (μ mol/L)	6.25 \pm 2.6	5.84 \pm 1.96	0.530
LDL-C (mg/dl)	103.02 \pm 24.88	111.11 \pm 35.43	0.350
HDL-C (mg/dl)	60.76 \pm 10.76	74.28 \pm 17.82	0.001**
TG (mg/dl)	273.88 \pm 94.3	237.72 \pm 83.4	0.160
Total-C (mg/dl)	220.32 \pm 31.02	233.04 \pm 38.39	0.200
Irisin (ng/ml)	39.2 \pm 28.55	69.06 \pm 96.62	0.015*

* $p < 0.05$; ** $p < 0.01$ statistical significance.

GDM – gestational diabetes mellitus; BMI – body mass index; HbA1c – glycosylated hemoglobin; FBG – fasting blood glucose; FI, fasting insulin; HOMA-IR, homeostasis model assessment of insulin resistance; CRP – c-reactive protein; TSH- Thyroid stimulated hormone; ALT-Alanine aminotransferase; AST-Aspartate aminotransferase; LDL-C, low density lipoprotein-cholesterol; HDL-C, high density lipoprotein-cholesterol; TG, total triglyceride.

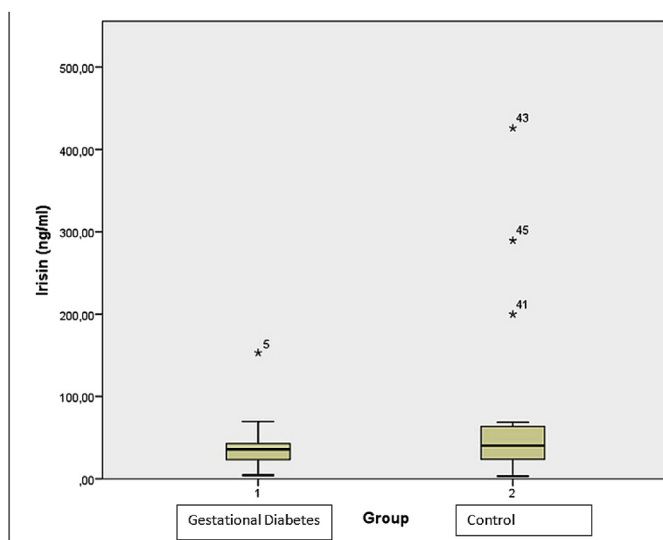


Fig. 1. Irisin level between GDM and control groups.

insulin resistance [5] and T1DM patients [8]. In most of these studies, insulin metabolism has been shown to be effective in the variation of irisin levels. A positive association between circulating irisin and markers of glucose intolerance has been shown by two independent studies in non-pregnant subjects [9,10]. Furthermore, irisin serum levels are lower in Chinese patients with new-onset T2DM as compared to non-diabetic subjects [6]. It has been suggested that lower levels of circulating irisin is associated with diabetes itself or the metabolic condition that caused progression to T2DM. Pregnancy might be another important factor for blood irisin. Kuzmicki et al. found that serum irisin increased significantly in pregnant women, but this increase was significantly lower in subjects with GDM [11]. In the present study, circulating irisin levels are significantly lower in women with GDM, and these data are consistent with the available data.

Low blood levels of irisin are closely related to BMI, fat mass, insulin resistance, and diabetes. The effects of irisin on energy metabolism is controversial. Stengel et al. have reported positive correlations between circulating irisin levels and BMI in people without diabetes [10]. In contrast, a study suggested that circulating irisin correlated negatively with BMI, waist–hip ratio and fat mass in men. Circulating irisin was still lower in overweight and obese men without diabetes [12]. Our study showed that maternal serum irisin levels were negatively correlated with BMI but positive correlated with insulin resistance (Table 2).

Since the discovery of irisin, research groups have tried to clarify its relationship with pregnancy. Garces et al. demonstrated the placental expression of irisin precursor protein (FNDC5) with increased serum levels with advancing gestation compared with non-pregnant women. Consistent with our findings, serum irisin levels were found to be positively correlated with HOMA-IR during pregnancy [13]. The results of studies on irisin and glucose homeostasis have been controversial. Irisin levels have also been negatively associated with HOMA-IR in some studies [11], and positively in others [14]. These contradictory outcomes may be explained by differences in the gestational age in the sampling period and the criteria used in screening for GDM.

Timmons JA et al. reported that myocyte expression of irisin was not related to markers of energy metabolism including BMI, fasting insulin and FBG [15]. However, Huh JY et al. found that circulating irisin concentrations were positively correlated BMI and FBG [9]. In

Table 2

Irisin level in GDM patients and the association between clinical and laboratory findings.

Variables	Irisin Univariate correlations		Irisin Multivariate regression analysis	
	r	p-value	β	p-value
Age	−0.454	0.023*	−4.814	0.295
Gravida	−0.139	0.508	6.750	0.047*
BMI	0.031	0.882	−5.613	0.551
HbA1c	−0.095	0.651	−18.223	0.389
FBG	−0.099	0.638	0.723	0.553
Glucose 1 h	0.018	0.930	0.271	0.669
Glucose 2 h	−0.606	0.001**	−1.176	0.814
FI	0.197	0.345	−1.264	0.348
HOMA-IR	0.225	0.279	10.446	0.709
Creatinine	−0.118	0.575	−54.416	0.665
CRP	0.006	0.977	−0.037	0.639
TSH	−0.192	0.358	−15.185	0.955
ALT	0.027	0.900	−0.144	0.301
AST	−0.010	0.963	0.646	0.898
Homocysteine	−0.204	0.328	8.915	0.808
LDL-C	0.104	0.621	−3.072	0.231
HDL-C	0.203	0.331	−0.792	0.203
TG	0.115	0.584	−0.265	0.418
Total-C	0.237	0.045*	2.238	0.392

*p < 0.05; **p < 0.01 statistical significance.

GDM – gestational diabetes mellitus; BMI – body mass index; HbA1C – glycosylated hemoglobin; FBG – fasting blood glucose; FI, fasting insulin; HOMA-IR, homeostasis model assessment of insulin resistance; CRP – c-reactive protein; TSH- Thyroid stimulated hormone; ALT-Alanine aminotransferase; AST-Aspartate aminotransferase; LDL-C, low density lipoprotein-cholesterol; HDL-C, high density lipoprotein-cholesterol; TG, total triglyceride.

the present study Our data showed that circulating irisin was negatively correlated with BMI and Glucose 2 h, but positively correlated with TC, TG, LDL-cholesterol (Table 2).

Depending on these findings we can speculate that, treatment strategies before a significant decrease in irisin levels may be more effective on reversing or stabilizing progression of prediabetes. Our data extending the results of previous studies demonstrated that T2DM is accompanied by lower circulating irisin. This would emphasize the ability of irisin to replenish glucose metabolism until metabolic failure reaches a point and would make irisin a useful parameter in metabolic research. We hypothesized that treatment modalities to increase irisin levels may be more beneficial before GDM develops. Clearly more work is needed to elucidate differences in the association between irisin on one hand and markers of the metabolic syndrome on the other hand described in the different studies.

The main limitation of our study is its cross-sectional design that does not allow us to establish a cause-effect relationship and the directionality of the results. Secondly; our study was conducted in a specific population, and it cannot be assured that the same results would be obtained in other ethnic or study groups.

In conclusion this study confirmed lower levels of circulating irisin in subjects with GDM. Further investigations are needed to explore the underlying biological effects of irisin on pregnant women.

Conflict of interest

The authors declare that they have no conflict of interest.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 Informed consent was obtained from all patients for being included in the study.

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Financial disclaimers

None.

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