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Association between plasma irisin in pregnancy and postpartum glucose levels among Chinese women: A cohort study

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ABSTRACT

Aims/Introduction: The association between plasma irisin and glucose levels in the general population is controversial, and few studies have longitudinally detected this correlation. We aimed to examine whether irisin in pregnancy was associated with postpartum glucose levels among Chinese women and explore the modifiable factors.

Materials and Methods: We carried out a prospective cohort study in Guangzhou, China, during 2017 and 2018, and 453 pregnant women (20–28 weeks) were enrolled. Plasma irisin levels in pregnancy were tested. At 6–8 weeks after delivery, 93 women with gestational diabetes mellitus (GDM) underwent a 75-g oral glucose tolerance test, and the other 360 women had a fasting blood glucose (FBG) test. Multivariable linear, quantile and logistic regressions were carried out.

Results: The mean plasma irisin in mid-pregnancy was 13.73 ng/mL. We observed a significantly negative association between mid-pregnancy irisin and postpartum FBG (β: −0.056 ± 0.024). However, quantile regression showed the association was only significant in high percentiles of FBG levels (P_{50} to P_{95}), and the magnitude showed an increasing trend. Higher baseline irisin was also associated with a lower risk of postpartum impaired fasting glucose (relative risk 0.563, 95% confidence interval 0.384–0.825). Furthermore, we found significant interactions between irisin and predominant breast-feeding on FBG and impaired fasting glucose (both $P_{interaction} < 0.05$). In women with GDM, baseline irisin was non-significantly associated with postpartum postprandial 2-h glucose levels (β: −0.305 ± 0.160, $P = 0.061$).

Conclusions: Plasma irisin levels in mid-pregnancy were negatively associated with FBG levels and impaired fasting glucose at 6–8 weeks postpartum among Chinese women, and stronger associations were observed in women with higher FBG values. Furthermore, breast-feeding might modify this relationship.

INTRODUCTION

Pregnancy is a complicated physiological challenge that requires adaptations in multiple systems, and of great importance is the maintenance of glucose homeostasis [1]. Generally, postpartum glucose levels are expected to return to pre-gravid status, but women might also develop persistent glucose metabolism disorders, which include impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and type 2 diabetes mellitus at early postpartum[2]. Previous studies showed that postpartum women with the isolated IFG, isolated IGT or combined IFG and IGT showed a higher risk of developing type 2 diabetes mellitus [3-4]. Improving glucose homeostasis and expenditure at early postpartum might prevent or delay the onset of type 2 diabetes mellitus[2,5]. Therefore, it is imperative to discover maternal factors related to glucose homeostasis, to develop possible preventive strategies for postpartum glucose disorders. Myokines,

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secreted from maternal muscle tissues, is one of the factors that contribute to metabolic homeostasis [6]. Irisin is a newly identified myokine that regulates glucose metabolism [7].

Boström *et al.*[7] introduced the myokine, irisin, as an exercise-inducible secreted factor that improved glucose tolerance and increased energy expenditure in mice. Other experimental studies also showed that irisin might improve glucose homeostasis by reducing insulin resistance and promoting β-cell survival[8-10]. Subsequently, population-based studies have shown lower irisin levels in individuals with type 2 diabetes mellitus[11-13], and some concluded a negative association between irisin and fasting blood glucose (FBG) [11,14-15]. However, other literature reported a null[16] or even positive[17-18] correlation. Taken together, no consensus exists regarding the relationship between irisin and glucose levels in epidemiological studies.

Furthermore, several additional gaps remain in the existing literature. First, previous studies, which explored the aforementioned associations, focused on the general population or patients with metabolic diseases, such as type 2 diabetes mellitus or obesity[19]. While no studies paid attention to women at early postpartum. Furthermore, most studies were cross-sectional or retrospective designs, which lacked longitudinal data to verify the cause–effect relationship between irisin and glucose levels [19-20].

Thus, in consideration of the importance of glucose homeostasis for postpartum women, we carried out a prospective cohort study to examine the association between irisin in pregnancy and postpartum glucose levels and IFG among Chinese women, and explore the modifiable factors.

MATERIALS AND METHODS

Study design and participants

The present study was a prospective cohort study (registration number: NCT03023293) carried out at a hospital in Guangzhou, China during 2017 and 2018. For baseline examination, we enrolled pregnant women at 20–28 weeks of gestation. At 6–8 weeks after delivery, women returned to the hospital for follow up and re-examination. This study was approved by the institutional review boards of Sun Yat-sen University. Informed consent was obtained from all participants.

The inclusion criteria were: (i) aged 20–45 years; (ii) registered to deliver in this hospital; and (iii) blood samples were taken. Women with a history of reported pre-existing diabetes mellitus, cardiovascular disease, thyroid disease, hematopathy, polycystic ovary syndrome, infection during pregnancy, mental disorders or multiple gestation were excluded before enrollment. A total of 679 pregnant women were recruited at baseline, and 494 women returned at 6–8 weeks postpartum for follow up. We further excluded those who had implausibly high (>60 ng/mL) plasma irisin level (n = 1) in the baseline examination, or did not undergo glucose tests (n = 40) at postpartum. Therefore, 453 women were finally included in our analysis, of whom 93 were patients with gestational diabetes mellitus (GDM).

Measurement of plasma irisin in baseline

In baseline, blood samples for circulating irisin were collected by experienced nurses in the morning after an overnight fast for at least 10 h. These blood samples were then centrifuged at 2,014 g for 15 min and processed for plasma samples, which were then stored at − 80°C for the following assays. Plasma irisin concentrations were determined using an enzyme-linked immunosorbent assay (EK-067–29; Phoenix Pharmaceuticals, Burlingame, CA, USA) with a spectrophotometric microplate reader at a wavelength of 450 nm (Spark® multimode microplate reader, Tecan Trading AG, Männedorf, Switzerland). The test provided a range of detection of 0.1–1,000 ng/mL, and showed an inter- and intra-assay coefficient of variation of <10 and 15%, respectively.

Postpartum glucose test and definitions

For follow up, participants with GDM (n = 93) were scheduled for a 75-g oral glucose tolerance test (OGTT), including OGTT-0 h glucose (FBG), OGTT-1 h and OGTT-2 h glucose, and the others (n = 360) underwent fasting glucose tests at 6–8 weeks after delivery. Glucose levels were measured using an automatic biochemical analyzer (BS-380; Mindray, Shenzhen, China) in a standardized clinical laboratory at the hospital.

Abnormal postpartum glucose levels were determined using the American Diabetes Association criteria for adults [21]. IFG was defined as a fasting glucose level of ≥5.6 mmol/L (100 mg/dL). IGT was defined as OGTT-2 h glucose level of ≥7.8 mmol/L (140 mg/dL). Fasting glucose levels of <5.6 mmol/L and 2-h blood glucose levels of <7.8 mmol/L were identified as normal.

Assessment of covariates

Questionnaire survey at baseline

Information on sociodemographic characteristics (e.g., age, occupation, education level and monthly household income), physical activity (PA), lifestyle factors in mid-pregnancy were obtained in baseline using self-reported questionnaires. The information of PA, which was expressed in metabolic equivalents (METs-min/week), was collected using the International Physical Activity Questionnaire [22], and participants were divided into two groups by the median (high and low) of METs. Participants' dietary intakes during the past month before enrollment were assessed using a validated 81-item quantitative food frequency questionnaire[23] in face-to-face interviews. Furthermore, family history of diabetes was also recorded.

All investigators were trained before the survey was administered. Each completed questionnaire was checked by an inspector.

Anthropometric information

Anthropometric measurements (e.g. height and pre-pregnancy weight) were taken by trained clinical nurses. Pre-pregnancy body mass index (BMI) was calculated as bodyweight in

kilograms divided by height in meters squared (kg/m^2). For stratified analyses, women were classified as thin/normal (<24 kg/m^2) or overweight/obese (≥24 kg/m^2) using the Chinese criteria [24].

Glucose test in baseline

In the baseline investigation, all pregnant women were scheduled a 75-g OGTT between 20–28 weeks of gestation. According to the criteria of the International Association of Diabetes and Pregnancy Study Groups[25], women were diagnosed as GDM based on the 75-g OGTT if they met at least one of the following criteria: FBG ≥5.10 mmol/L (92mg/dL); OGTT-1 h glucose ≥10.0 mmol/L (180 mg/dL); and OGTT-2 h glucose ≥8.50 mmol/L (153 mg/dL).

Postpartum information

At 6–8 weeks postpartum, we carried out face-to-face interviews with participants to obtain information of infant feeding patterns through the questionnaires. According to categories of the World Health Organization[26], the definition of predominant breast-feeding is as follows: infants received breast milk as the predominant source of nourishment, and they also have received liquids (water, and water-based drinks, fruit juice, oral rehydration salts), ritual fluids and drops or syrups (vitamins, minerals, medicines) in limited quantities. The participants were categorized into two subgroups according to predominant breast-feeding (yes v.s. no) for stratified analysis.

Statistical analysis

All statistical analyses were carried out using SAS statistical software package (version 9.4; SAS Institute Inc., Cary, NC, USA). Continuous variables were reported as the mean ± standard deviation (SD), and categorical variables were expressed as percentages. Differences between groups were evaluated by Student's *t*-test or χ^2-tests.

Multivariable linear and logistic regression analyses were carried out to evaluate the associations between plasma irisin in pregnancy and postpartum glucose levels and IFG/IGT. Model 1 was not adjusted; model 2 was adjusted for maternal age, pre-pregnancy BMI and PA in mid-pregnancy; model 3 was additionally adjusted for predominant breast-feeding at postpartum, family history of diabetes, passive smoking, energy intake, energy proportion of fat, protein and carbohydrates, and fasting glucose levels in mid-pregnancy; model 4 was further adjusted for educational level, monthly household income and occupation. We stratified the participants by age, pre-pregnancy BMI, PA in mid-pregnancy, history of GDM and predominant breast-feeding, respectively, and investigated the interactions between irisin and stratified factors on FBG/IFG.

To identify how the levels (low and high quantiles) of FBG were associated with irisin, a multivariate quantile regression was carried out[27-28]. We carried out quantile regression models using postpartum FBG as an outcome variable and irisin levels as predictors while adjusted for some covariates in model 4, assessing the 0.05, 0.25, 0.50, 0.75 and 0.95 quantiles of the FBG distribution. All the tests were considered to be statistically significant if $P < 0.05$.

RESULTS

Participant characteristics

The general characteristics of the participants are shown in Table 1. A total of 453 women was included in the study, of whom 93 (20.53%) were diagnosed as GDM. The prevalence of postpartum IFG in the total population is 14.35%. Among women with a history of GDM, 35.48% had postpartum abnormal glucose regulation (IGT or IFG), whereas 24.73% had postpartum IGT and 16.13% had postpartum IFG. Participants had a mean age of 30.6 years (SD 4.7 years) at baseline. The mean plasma irisin level during mid-pregnancy was 13.7 ng/mL (SD 5.9 ng/mL), and irisin levels were significantly lower in women with IFG than the normal-glucose group (12.0 vs 14.0 ng/mL). Meanwhile, pre-pregnancy BMI and mid-gestation FBG were significantly higher in the IFG group (both $P = 0.002$). Women without IFG had a higher percentage of predominant breast-feeding (50.42 vs 28.81%). No significant differences were observed in socioeconomic characteristics and dietary nutrients intakes between groups ($P > 0.05$).

Associations between plasma irisin in pregnancy and postpartum glucose levels

As shown in Table 2, circulating irisin in mid-pregnancy was negatively correlated with postpartum FBG levels (β: −0.056 ± 0.024) after adjustment for covariates. Among women with previous GDM, the significantly inverse association between plasma irisin and postpartum OGTT-2 h glucose levels was found in model 3 (β: −0.305 ± 0.152, $P = 0.049$), whereas this association became non-significant ($P = 0.061$) after further adjusting for sociodemographic characteristics. Among women with a history of GDM, no significant associations were found between mid-pregnancy irisin and postpartum postprandial 1-h glucose during OGTT.

Association between irisin and FBG by quantile regression

The results of quantile regression are presented in Figure 1. There were significantly negative associations of irisin levels with postpartum FBG in high percentiles (P_{50} to P_{95}), and the magnitude of this association showed an increasing trend. The data of FBG percentiles and coefficients of quantile regression were presented in Table S1.

Logistic regression of irisin levels in pregnancy with postpartum glucose status

As shown in Table 3, the relative risk (RR) for IFG was reduced by 43.7% per 1-SD increase in the irisin concentrations (RR 0.563; 95% confidence interval [CI] 0.384–0.825) after adjustment for covariates. Whereas no significant association was found between mid-pregnancy irisin levels and postpartum IGT.

Table 1 | Baseline and follow-up characteristics of participants

	Overall (n = 453)	Postpartum IFG		
		Yes (n = 65)	No (n = 388)	P-values
Baseline characteristics in mid-pregnancy				
Maternal age (years)	30.6 (4.7)	30.5 (4.6)	30.6 (4.7)	0.854
<35	81.46%	84.62%	80.93%	0.479
≥35	18.54%	15.38%	19.07%	
Pre-pregnancy BMI (kg/m^2)	20.7 (2.8)	21.9 (3.3)	20.5 (2.7)	0.003*
Normal or thin	85.39%	73.02%	87.47%	0.003*
Overweight or obese	14.61%	26.98%	12.53%	
Plasma irisin (ng/mL)	13.7 (6.0)	12.0 (4.7)	14.0 (6.1)	0.002*
FBG (mmol/L)	4.4 (0.4)	4.6 (0.3)	4.4 (0.4)	0.002*
OGTT 1-h glucose (mmol/L)	7.8 (1.8)	7.9 (1.6)	7.8 (1.8)	0.615
OGTT 2-h glucose (mmol/L)	6.7 (1.3)	6.9 (1.3)	6.7 (1.4)	0.487
GDM (yes)	20.53%	23.08%	20.10%	0.583
Family history of diabetes (yes)	16.81%	20.00%	16.28%	0.458
Occupation				
Housewives	24.72%	30.77%	23.71%	0.162
Administrators and clerks	25.17%	29.23%	24.48%	
Commerce and services	27.59%	27.69%	27.58%	
Other	22.52%	12.31%	24.23%	
Educational level				
Senior high school or below	33.11%	36.92%	32.47%	0.334
Junior collage	32.67%	36.92%	31.96%	
Collage or above	34.22%	26.15%	35.57%	
Monthly household income				
<4,000 RMB/person	19.21%	21.54%	18.81%	0.221
4,001–6,000 RMB/person	24.50%	26.15%	24.23%	
6,001–10,000 RMB/person	24.06%	30.77%	22.94%	
>10,000 RMB/person	32.23%	21.54%	34.02%	
Passive smoking (yes)	24.04%	25.81%	23.75%	0.725
Physical activity (METs-min/week)	1897.1 (1689.5)	1783.2 (1364.7)	1905.6 (1782.5)	0.522
High	49.89%	55.38%	48.97%	0.338
Low	50.11%	44.62%	51.03%	
Energy intake in mid-pregnancy				
Total energy	1818.7 (504.6)	1859.6 (525.5)	1812.1 (501.5)	0.492
Fat (g/day)	74.5 (20.1)	75.8 (20.3)	74.3 (20.1)	0.588
% Energy	0.4 (0.1)	0.37 (0.06)	0.37 (0.06)	0.957
Protein (g/day)	73.3 (27.3)	73.3 (28.9)	73.3 (27.0)	0.991
% Energy	0.2 (0.0)	0.15 (0.03)	0.16 (0.03)	0.170
Carbohydrates (g/day)	219.2 (71.9)	227.0 (76.1)	217.9 (71.2)	0.357
% Energy	0.5 (0.1)	0.48 (0.07)	0.48 (0.06)	0.475
Postpartum glucose levels				
FBG (mmol/L)	5.2 (0.5)	6.0 (0.5)	5.0 (0.4)	<0.001*
OGTT 1-h glucose (mmol/L)†	8.8 (1.6)	9.2 (1.5)	8.7 (1.6)	0.248
OGTT 2-h glucose (mmol/L)†	6.9 (1.5)	7.3 (1.8)	6.9 (1.4)	0.330
IGT (yes)†	24.73%	33.33%	23.08%	0.384
Predominant breast-feeding at postpartum				
Yes	47.36%	28.81%	50.42%	0.002*
No	52.64%	71.19%	49.58%	

Total n = 453. *Significant results (P < 0.05). †Postpartum oral glucose tolerance test (OGTT) was carried out and impaired glucose tolerance (IGT) was diagnosed among participants with a history of gestational diabetes mellitus (GDM), n = 93 (20.53% out of 453 women). %Energy, energy proportion of protein, fat or carbohydrates, respectively; BMI, body mass index; FBG, fasting blood glucose; IFG, impaired fasting glucose; MET, metabolic equivalent.

Table 2 | Multivariate linear regression analysis between irisin level in mid-pregnancy and postpartum glucose

	Postpartum fasting glucose (n = 453)		Postpartum OGTT-1 h glucose (n = 93)†		Postpartum OGTT-2 h glucose (n = 93)†	
	beta (SE)	P	beta (SE)	P	beta (SE)	P
Model 1	−0.070 (0.024)	0.004*	−0.172 (0.139)	0.220	−0.254 (0.132)	0.059
Model 2	−0.072 (0.024)	0.003*	−0.169 (0.154)	0.279	−0.277 (0.146)	0.061
Model 3	−0.056 (0.024)	0.018*	−0.099 (0.162)	0.545	−0.305 (0.152)	0.049*
Model 4	−0.056 (0.024)	0.019*	−0.090 (0.170)	0.598	−0.305 (0.160)	0.061

*Significant results ($P < 0.05$). Model 1 was not adjusted; model 2 was adjusted for maternal age, pre-pregnancy body mass index and physical activity in mid-pregnancy; model 3 was further adjusted for predominant breast-feeding at postpartum, family history of diabetes, passive smoking, energy intake, energy proportion of fat, energy proportion of protein, energy proportion of carbohydrates and fasting glucose levels in mid-pregnancy; model 4 was further adjusted for educational level, monthly household income and occupation. †Postpartum oral glucose tolerance test was carried out among participants with history of gestational diabetes mellitus, n = 93. SE, standard error.

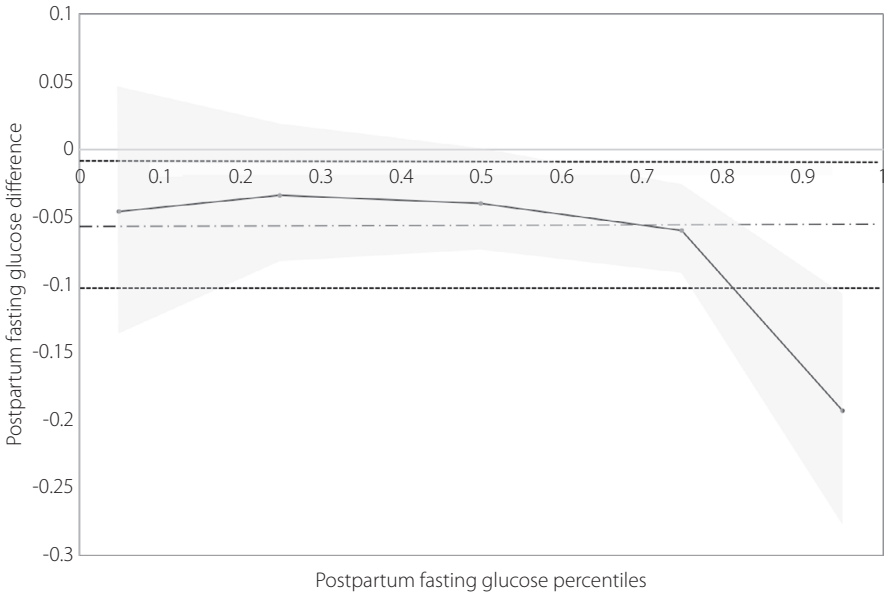

Figure 1 | Point estimates and 95% confidence bounds (grey area) for quantile regression between irisin in mid-pregnancy and postpartum fasting glucose levels. The dots represent specific glucose percentiles (0.05 percentile, 0.25 percentile, 0.50 percentile, 0.75 percentile and 0.95 percentile) in the multivariable (adjusted) quantile regression model. The horizontal dashed line represents the linear regression coefficient and its respective confidence intervals. Model was adjusted for maternal age, pre-pregnancy body mass index, physical activity in mid-pregnancy, predominant breast-feeding at postpartum, family history of diabetes, passive smoking, energy intake, energy proportion of fat, energy proportion of protein, energy proportion of carbohydrates, fasting glucose levels in mid-pregnancy, educational level, monthly household income and occupation.

Association between irisin and FBG/IFG by subgroups

We stratified the population by age, pre-pregnancy weight status, mid-pregnancy PA, history of GDM and predominant breast-feeding, respectively (Table 4 and Table S2). We found that irisin in mid-pregnancy was negatively associated with postpartum IFG in women with predominant breast-feeding, but not in their counterparts, and there were statistically significant interactions between irisin and predominant breast-feeding on postpartum FBG and IFG ($P_{interaction}$ = 0.005, 0.013, respectively). Furthermore, we observed significantly inverse correlations between irisin in mid-pregnancy and postpartum IFG in women aged <35 years, with higher PA, and without history of GDM, but not in their counterparts. However, no significant interactions were found between irisin and age, pre-pregnancy BMI, mid-pregnancy PA, and history of GDM on FBG or IFG.

DISCUSSION

To the best of our knowledge, this is the first longitudinal study to explore whether irisin in pregnancy was associated with postpartum glucose levels and IFG, and we found a significantly negative relationship between them. The magnitude of this association showed an increasing trend along with the

Table 3 | Logistic regression analysis of irisin level in mid-pregnancy with postpartum glucose status

	Postpartum IFG (n = 453)		Postpartum IGT (n = 93)†	
	RR (95% CI)	P	RR (95% CI)	P
Model 1	0.678 (0.504–0.911)	0.010*	0.832 (0.530–1.308)	0.426
Model 2	0.649 (0.477–0.882)	0.006*	0.830 (0.521–1.324)	0.434
Model 3	0.564 (0.388–0.820)	0.003*	0.845 (0.431–1.657)	0.625
Model 4	0.563 (0.384–0.825)	0.003*	0.911 (0.438–1.893)	0.802

*Significant results (P < 0.05). Model 1 was not adjusted; model 2 was adjusted for maternal age, pre-pregnancy body mass index and physical activity in mid-pregnancy; model 3 was further adjusted for predominant breast-feeding at postpartum, family history of diabetes, passive smoking, energy intake, energy proportion of fat, energy proportion of protein, energy proportion of carbohydrates and fasting glucose levels in mid-pregnancy; model 4 was further adjusted for educational level, monthly household income and occupation. †Postpartum impaired glucose tolerance (IGT) was diagnosed among participants with a history of gestational diabetes mellitus, n = 93. CI, confidence interval; IFG, impaired fasting glucose; RR, relative risk.

Table 4 | Association between irisin and impaired fasting glucose in subgroups

	Postpartum IFG (n = 453)		
	RR (95% CI)	$P^{\dagger}$	P_{inter}
Age			
≥35	0.132 (0.015 – 1.175)	0.069	0.101
<35	0.593 (0.398 – 0.884)	0.010*	
Pre-pregnancy BMI			
Normal or thin	0.579 (0.388 – 0.880)	0.011*	0.500
Overweight or obese	0.350 (0.127 – 0.961)	0.042*	
Physical activity			
High	0.373 (0.192 – 0.724)	0.004*	0.656
Low	0.671 (0.414 – 1.089)	0.106	
GDM			
Yes	0.621 (0.273 – 1.416)	0.258	0.873
No	0.521 (0.332 – 0.816)	0.004*	
Predominant breast-feeding			
Yes	0.349 (0.156 – 0.783)	0.011*	0.013*
No	0.711 (0.448 – 1.126)	0.145	

*Significant results (P < 0.05). †Models were adjusted for maternal age, pre-pregnancy body mass index (BMI), physical activity in mid-pregnancy, predominant breast-feeding at postpartum, family history of diabetes, passive smoking, energy intake, energy proportion of fat, energy proportion of protein, energy proportion of carbohydrates, fasting glucose levels in mid-pregnancy, educational level, monthly household income and occupation. CI, confidence interval; GDM, gestational diabetes mellitus; IFG, impaired fasting glucose; RR, relative risk.

increase of postpartum FBG levels (from P_{50} to P_{95}). We also found that higher irisin levels in pregnancy were associated with a lower risk of postpartum IFG. The present study also provided new evidence that there were significant interactions between plasma irisin and predominant breast-feeding on FBG levels and IFG.

Although the association between plasma irisin in pregnancy and postpartum FBG has not been reported previously, several studies have observed negative associations between irisin and FBG in other populations[11-12,14-15,29]. For instance, Zhao *et al.*[14] reported that irisin levels were negatively correlated with FBG in pregnant women, of whom 50% were patients with GDM. Similarly, another study carried out in Korea reported a negative association between irisin and FBG in individuals with new-onset type 2 diabetes mellitus [11]. Furthermore, irisin was inversely correlated with FBG in Bulgarian adults, 37.5 and 31.3% of whom were patients with prediabetes and type 2 diabetes mellitus, respectively[15]. The present finding of a negative association between plasma irisin and FBG is consistent with these aforementioned studies.

However, there is some contradictory evidence that described a positive[17-18] association between irisin and glucose levels. Huh *et al.*[17] and De Meneck *et al.*[18] reported positive associations between irisin and FBG in healthy and obese individuals without diabetes. Another meta-analysis suggested that circulating irisin was positively associated with insulin resistance in adults without diabetes[30]. A potential explanation for the discrepant findings is various metabolic status of the participants, and irisin levels were associated with physiological status [31]. Consistent with this viewpoint, the present results of quantile regression showed that there were considerably stronger associations between mid-pregnancy irisin and postpartum FBG in women with higher glucose levels compared with normoglycemic women. Hyperglycemia might be accompanied by abnormal metabolic status, including insulin resistance, and individuals might develop diabetic complications along with other metabolic disorders [32]. The stronger association in women with higher FBG might suggest that irisin played an important role in glucose regulation, especially in participants with glucose disorders. Furthermore, another explanation for the discrepant findings might be ethnic variability. A meta-analysis reported that the association between irisin and insulin resistance was more pronounced in people from Asia and North America, but not in Europeans[30].

Furthermore, we also observed that higher irisin concentrations were related to a decreased risk of IFG, which was in accordance with previous studies. Yan *et al.*[33] reported that irisin was significantly associated with a reduced risk of raised FBG. Liu *et al.*[34] found significantly lower irisin levels in type 2 diabetes mellitus patients compared with healthy controls; and lower serum irisin was also found in new-onset[11] and undefined type 2 diabetes mellitus patients[35], respectively. These findings support our hypothesis that elevated irisin levels during pregnancy can likely reduce the risk of postpartum IFG.

Previous literature with regard to the relationship between irisin and postprandial glucose was controversial. Some

reported a negative association between irisin and postprandial glucose in patients with type 2 diabetes mellitus[11,36-37]. Whereas a Korean study that included adults with and without type 2 diabetes mellitus reported a positive association between them[38]. In the present study, we explored the association between mid-pregnancy irisin and postpartum postprandial glucose levels in women with GDM (n = 93), and we found a non-significant negative association between irisin levels and postpartum postprandial 2-h glucose levels. The present results might be explained by the low number of participants with a history of GDM and a lack of statistical power. Meanwhile, postprandial glucose, compared with FBG, might be influenced by external factors, such as energy intakes and gastrointestinal absorption function [39-40], which might lead to the discrepant results.

Several underlying mechanisms linking irisin to glucose homeostasis, as well as diabetes, were suggested as follows. First, emerging evidence has shown that recombinant irisin in animal models or *in vitro* irisin treatment in cell culture systems is associated with improved glycemic control and ameliorated insulin resistance[10,41]. Second, irisin was shown to stimulate glucose uptake through adenosine monophosphate-activated protein kinase activation in muscle cells[41-42]. Also, irisin reduced hepatic glucose output (gluconeogenesis), but increased glycogen content (glycogenesis) through adenosine monophosphate-activated protein kinase pathway[43-44]. In addition, irisin stimulates browning of white adipocytes and induces the expression of mitochondrial uncoupling protein-1[45-46], which increases energy expenditure and thermogenesis[7]. The aforementioned stimulation was probably by activation of the p38 mitogen-activated protein kinase and extracellular signal-regulated kinase signaling pathway[47]. Accordingly, it can be speculated that the inverse relationship between plasma irisin and glucose levels was biologically plausible.

Additionally, we also carried out stratified analysis by subgroups to explore the modifiable factors for the association between irisin and glucose levels. Interactions between irisin and predominant breast-feeding on FBG and IFG were both significant. It is well established that breast-feeding might may reduce maternal hyperglycemia[48-49] and the likelihood of type 2 diabetes mellitus later in life[50]. Gunderson *et al.*[49] reported the preventive effect of breast-feeding against the development of prediabetes and diabetes during the early postpartum period in the Study of Women, Infant Feeding and Type 2 Diabetes (SWIFT) cohort. Similarly, a review reported that breast-feeding was a modifiable factor on postpartum glucose levels[2].

The present study is the first to detect the modifying effect of predominant breast-feeding on the association of irisin and FBG. Nevertheless, consistent with previous studies[51-52], we observed a non-significant interaction between irisin and PA in pregnancy on postpartum glucose levels. The interactions between irisin and age, pre-pregnancy BMI and history of GDM were also non-significant. Further studies are required to validate the present findings and elucidate the potential mechanisms that regulate irisin activity.

In particular, the significantly negative associations between irisin during pregnancy with postpartum FBG and IFG were robust, even after adjustment for glucose levels in mid-pregnancy. A systematic review concluded that the direct effects of irisin on glucose regulatory mechanisms in different organs might contribute to postpartum normoglycemia[53]. Furthermore, we discovered that a relatively high proportion of women without a history of GDM developed IFG in early postpartum (13.89%). Therefore, the present findings have clinical implications that plasma irisin in pregnancy might be a predictive factor of postpartum FBG and IFG in women with or without a history of GDM.

The present study had several limitations. First, there was some loss to follow up, which might result in selection bias in this study. However, as shown in Table S3, no significant difference was found in the distribution of age, BMI or other characteristics between participants who returned for postpartum evaluation and those who did not return, indicating that selection bias originating from non-participation might be small. Second, although we adjusted for some important characteristics, we could not completely exclude residual confounding by unmeasured potential confounders. Third, the follow-up period to 6–8 weeks postpartum might not be long enough for estimating the incidence of glucose metabolic disorders. However, these results are still valuable for understanding the current extent of glucose disorders for postpartum women. Further long-term longitudinal studies are warranted to provide more insights into the nature of the association. Finally, the small sample size of women who underwent an OGTT at postpartum might weaken the statistical power of the correlation. External, larger-scale validation is therefore necessary in future studies.

The present study showed that circulating irisin levels during pregnancy were negatively associated with FBG levels at 6–8 weeks postpartum, and higher irisin concentrations were related to a decreased risk of IFG. Stronger associations were observed in women with higher postpartum FBG levels. In addition, breast-feeding might be a modifiable factor of this association.

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DISCLOSURE

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 | Regression coefficients and 95% confidence intervals of irisin levels during mid-pregnancy and postpartum fasting glucose percentiles.

Table S2 | Multivariate linear regression analysis between irisin level in mid-pregnancy and postpartum fasting glucose by subgroups.

Table S3 | Comparison of participants who returned for postpartum follow up versus defaulters.