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The association between circulating phenylalanine and the temporal risk of impaired insulin markers in gestational diabetes mellitus

Hao Wu [a], Qiong Wang [b], Yanmin Chen [b], Danqing Chen [b,*]

[a] Department of Obstetrics Central Laboratory, Women's Hospital School of Medicine Zhejiang University, China
[b] Department of Maternity Inpatient, Women's Hospital School of Medicine Zhejiang University, China

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ABSTRACT

Background: We aimed to contrast plasma amino acid concentrations in pregnant women with Gestational Diabetes Mellitus (GDM) to those without, to analyze the link between plasma amino acid concentrations, GDM, insulin resistance, and insulin secretion at 24–28 weeks of gestation.
Methods: The research employed a retrospective case-control study design at a single center. Basic demographic and laboratory data were procured from the hospital's case system. The study encompassed seventy women without gestational diabetes mellitus (GDM) and thirty-five women with GDM matched in a 1-to-2 ratio for age and pre-pregnancy BMI. Utilizing high-performance liquid chromatography-mass spectrometry (HPLC-MS), peripheral fasting plasma amino acid concentrations in these women, during mid-pregnancy, were duly measured. We carefully evaluated the significant differences in the quantitative data between the two groups and developed linear regression models to assess the independent risk factors affecting insulin resistance and insulin secretion.
Results: Significant variations in insulin secretion and resistance levels distinguished GDM Group from the non-GDM group at three distinct time points, alongside relatively elevated serum Glycosylated Hemoglobin (HbA1c) levels. Triglycerides (TG) were also significantly increased in those with GDM during adipocytokine observations. Apart from glutamic acid and glutamine, the concentrations of the remaining 16 amino acids were notably increased in GDM patients, including all branched chain amino acids(BCAAs) and aromatic amino acids(AAAs). Ultimately, it was ascertained that fasting serum phenylalanine levels were independent risk factors affecting insulin resistance index and insulin secretion at various phases.
Conclusions: Various fasting serum amino acid levels are markedly increased in patients with GDM, specifically phenylalanine, which may play role in insulin resistance and secretion.

1. Introduction

Pregnancy induces macroscopic metabolic alterations designed to cater to fetal needs and prepare for the energy demands of the postpartum period [1]. A healthy pregnancy features reduced insulin sensitivity, which facilitates metabolic substrate availability for the fetus [2]. Nevertheless, an imbalance between insulin secretion and resistance can often instigate abnormal energy metabolism, primarily evidenced through glucose fluctuations, thereby leading to Gestational Diabetes Mellitus (GDM) [3,4]. The prevalence of GDM has been markedly increased in recent decades worldwide, largely attributed to enhanced energy resource availability, and displays regional and ethnic variations, with Asians particularly susceptible [5]. In China, GDM morbidity has reached 18% per available data [6,7]. GDM is commonly associated with various maternal and infant perinatal complications, including cystitis, gestational hypertension, hydramnios in mothers, and prematurity, hypoglycemia, macrosomia in infants [8,9]. Furthermore, women with a GDM history face an increased risk of developing type 2 diabetes mellitus (T2DM), metabolic syndrome, and cardiovascular disease [10]. Their offspring may also become significantly susceptible to impaired glucose tolerance, diabetes, obesity, and coronary heart disease [11].

Despite these findings, the pathogenesis of GDM remains elusive, hindering the development of effective preventive strategies and treatments. Pathological insulin resistance due to GDM often culminates in adverse pregnancy outcomes and severe long-term maternal metabolic complications [12,13]. Recent advancements in metabolomics, a holistic and emergent methodology, offer the potential to simultaneously analyze numerous metabolites and uncover novel molecular markers

* Corresponding author at: Women's Hospital School of Medicine Zhejiang University, 1 Xuxue Road, Shangcheng District, Hangzhou 310000, China.
E-mail address: chendq@zju.edu.cn (D. Chen).

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across various biological fields [14]. Metabolic studies concentrating on GDM have identified an association between abnormal glucose levels during pregnancy and irregular serum amino acid profiles [15,16], although results have been somewhat inconsistent, potentially due to variations in GDM diagnostic criteria, usage of different metabolomics instrumental methods, and divergences in biological specimens [17–20]. Several untargeted metabolomics studies have proposed that abnormally elevated amino acid levels are common in GDM. This is particularly true for branched-chain amino acids. However, the equally abnormal elevation of aromatic amino acids, correlating with blood glucose, serum insulin secretion levels, and insulin resistance index, has been relatively underexplored.

Consequently, we embarked on an analysis to illuminate the association between serum amino acid levels and insulin-related targets in pregnant women, including those with and without GDM. Employing a targeted high-performance liquid chromatography-mass spectrometry (HPLC-MS) metabolomics approach, we have made considerable progress in our current investigations.

2. Patients and methods

2.1. Sample collection

The study - conducted from 01.01.2017 to 31.12.2019 at the outpatient department of Women's Hospital School Of Medicine Zhejiang University- was a retrospective case-control investigation featuring pregnant women aged 24–45 years, in their 23-28th weeks of gestation, all of Han Chinese descent. Exclusion criteria encompassed obesity, preexisting type 1 or 2 diabetes, abnormal oral glucose tolerance test results at baseline, use of any medication (excluding Levothyroxine), multiple pregnancy, any acute or chronic inflammatory diseases during pregnancy, and smoking or heavy drinking habits. Out of 442 eligible pregnant women, 422 underwent a 75-g oral glucose tolerance test (OGTT) during their second trimester (24–28 weeks). GDM diagnoses were established if a single plasma glucose measurement met or exceeded the following thresholds: fasting level of 5.1 mmol/L, 1-h level of 10.0 mmol/L, or 2-h level of 8.5 mmol/L. Thereafter, 35 GDM-diagnosed women were matched with 70 healthy women based on maternal age, gestational age and pre-pregnancy body mass index categories. The study was conducted following the STROBE Statement, with the Ethics Committee of Hangzhou Women's Hospital's approval and informed consents from all participants.

2.2. Sample data and laboratory measurements

Clinical medical records provided anthropometric data (pre-pregnancy weight, weight gain, height, gestational age at sample collection, medication history, etc.). Following an overnight fast, three blood samples were drawn during the OGTT (fasting, 1-h, 2-h). All serum indices were processed at our clinical laboratory. Serum FBG, 1 h-OGTT, 2 h-OGTT, FINS, 1 h-INS, 2 h-INS, triglyceride, total cholesterol, HDL, LDL, HbA1c, and other biochemical indices were determined using a chemiluminescent microparticle immunoassay (UniCel DXI 800; Beckman Coulter Inc., Brea, CA, USA). Remaining serum samples were immediately stored at −80 °C for subsequent AA detection. The Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) and insulin resistance index were computed as follows: HOMA-IR = [fasting glucose (mmol/L) × fasting insulin (μIU/mL)]/22.5.[21]

2.3. AA metabolic analysis

A Thermo Scientific TSQ9000 GMS was used for qualitative and quantitative detection of target metabolites, employing specific analysis conditions and methods, including chromatographic conditions like DB-5MS capillary column, high-purity helium as the carrier gas, a flow rate of 1.2 mL/min, and a solvent delay of 4 min. The electron bombardment ion source temperature was 300 °C, with a transmission line temperature of 280 °C, selective reaction detection scanning (SRM) mode, and a mass scanning range of *m/z*: 40–600.

2.4. Statistical analysis

All quantitative variables were tested for normality. Normally distributed variables were represented by mean ± standard deviation (x ± s) or by quartile. Two-sample *t*-tests were used for normally distributed data, while Mann-Whitney *U* test was utilized for asymmetric distribution. Spearman correlation coefficients r and corresponding *p* values were used to assess the correlation between glucose, insulin parameters, and amino acid levels in each group, controlling for potential confounders like age, parity, and sampled BMI. Multiple linear regression analysis using stepwise regression controlled for age, gestational time, gestational age of sampling, while considering differences in BMI at sampling, triglyceride, HbA1c and amino acids as independent variables for insulin secretion and insulin resistance. All statistical analyses were conducted using SPSS, version 20.0, and $p < 0.05$ was deemed significant.

3. Result

3.1. Basic characteristics in pregnant women

In the onset, we screened enrolled gestational women and matched them with BMI and age parameters. Despite no significant variations in these criteria, we noticed substantial increases in BMI and weight gain during the first two trimesters among GDM patients compared to the control group(Table 1), who shared similar pregravidic BMI and weight baselines.

3.2. Clinical laboratory indicators of detection in mid-pregnancy

We conducted serum glucose and insulin testing at three time points (fasting, 1 h, 2 h). Simultaneously, additional laboratory tests revealed abnormally heightened serum insulin levels in GDM patients across the different time frames, with 1 h and 2 h insulin levels being especially prominent. Insulin resistance similarly increased, as evidenced by significantly elevated fasting glucose and fasting insulin levels in GDM individuals(Table 2). While lipid metabolism only showed a moderate surge in triglycerides, with no other abnormalities in the common lipid metabolism indices, differences in glycosylated hemoglobin were noteworthy. It was noted that glycosylated hemoglobin levels were significantly elevated in patients with gestational diabetes mellitus (GDM), mirroring the increase in blood glucose levels.

Table 1
Clinical characteristics of the GDM and control pregnant groups at 24–28 weeks of pregnancy.

Characteristics	Control group(n = 70)	GDM group(n = 35)	P value
Age(year)	32.27 ± 3.97	32.54 ± 4.92	0.761
Gravidity	2(1,3)	2(1,3)	0.497
Parity	0(0,1)	1(0,1)	0.509
Sample at gestational age (week)	25.24 ± 1.13	25.23 ± 1.49	0.962
Height(cm)	162.29 ± 5.27	161.26 ± 5	0.322
Pre-pregnacy BMI(kg/m^2)	19.89(18.77,21.91)	19.86 (18.75,21.45)	0.778
BMI at sampled(kg/m^2)	23.41(21.39,25.36)	24.17 (23.07,26.85)	**0.041***
Weight gain at sampled(kg)	9(6.15,10.13)	11(7,13.5)	**0.022***

Data are presented as mean ± SD. BMI, body mass index; *P* values in bold indicate significant differences (* < 0.05 ** < 0.01, *** < 0.001).

Table 2
Laboratory characteristics of the GDM and normal pregnant groups at 24–28 weeks of pregnancy.

Characteristics	Control group(n = 70)	GDM group(n = 35)	P value
FBG(mmol/L)	4.14(3.96,4.28)	4.52(4.28,5.14)	**0.001*****
1hOGTT(mmol/L)	7.49(6.59,8.54)	10.52(9.63,11.14)	**0.001*****
2hOGTT(mmol/L)	6.6(6.04,7.41)	8.82(8.1,9.38)	**0.001*****
FINS(μIU/mL)	7.78 ± 2.54	11.34 ± 5.07	**0.001****
1 h-INS(μIU/mL)	54.85(43.93,78.60)	87.10(55.30,111.00)	**0.001*****
2 h-INS(μIU/mL)	49.20(38.58,68.18)	71.30(50.50,91.70)	**0.001*****
HOMA-IR	1.43 ± 0.5	2.43 ± 1.22	**0.001*****
TG(mg/dL)	1.89(1.59,2.32)	2.22(1.83,3.392)	**0.008****
TC (mg/dL)	6.19 ± 0.83	6.3 ± 1.26	0.600
HDL (mg/dL)	2.02(1.86,2.37)	2.13(1.8,2.28)	0.978
LDL (mg/dL)	3 ± 0.63	3.02 ± 0.87	0.908
HbA1c(mmol/L)	4.89 ± 0.3	5.22 ± 0.39	**0.0001*****

Data are presented as mean ± SD. FBG, fasting blood glucose; OGTT, oral glucose tolerance test; tAUC, total area under curve; FINS, fasting insulin; INS, insulin; HOMA-IR, homeostatic model assessment of insulin resistance; TG, total cholesterol; TC, Total triglycerides; LDL, low-density lipoprotein; HDL, high-density lipoprotein; P values in bold indicate significant differences (* < 0.05 ** < 0.01, *** < 0.001).

3.3. Serum amino acid levels and correlation analysis in fasting women in mid-pregnancy

For fasting serum levels, all amino acids, except glutamic acid and glutamine, showed some degree of elevation - notably, the eight essential amino acids, all branched-chain amino acids and aromatic amino acids (Table 3). Within the branched-chain amino acid group, differences in isoleucine and valine were more distinct than that in leucine. Among the three aromatic amino acids, phenylalanine emerged the most pronounced. Upon adjusting for age, parity, and mid-pregnancy BMI, most amino acids with varying serum concentrations displayed positive correlations with both fasting insulin levels and glycemic indices (Fig. 1). However, the most compelling correlations were exhibited by aromatic and branched-chain amino acids (Table 4). This correlation, however, weakened for 1 h and 2 h glucose indices and for insulin resistance indices. Yet, the connection remained robust for 1 h and 2 h insulin and insulin resistance, where aromatic amino acids' correlation with insulin resistance and secretion was marginally stronger than branched-chain amino acids, particularly phenylalanine.

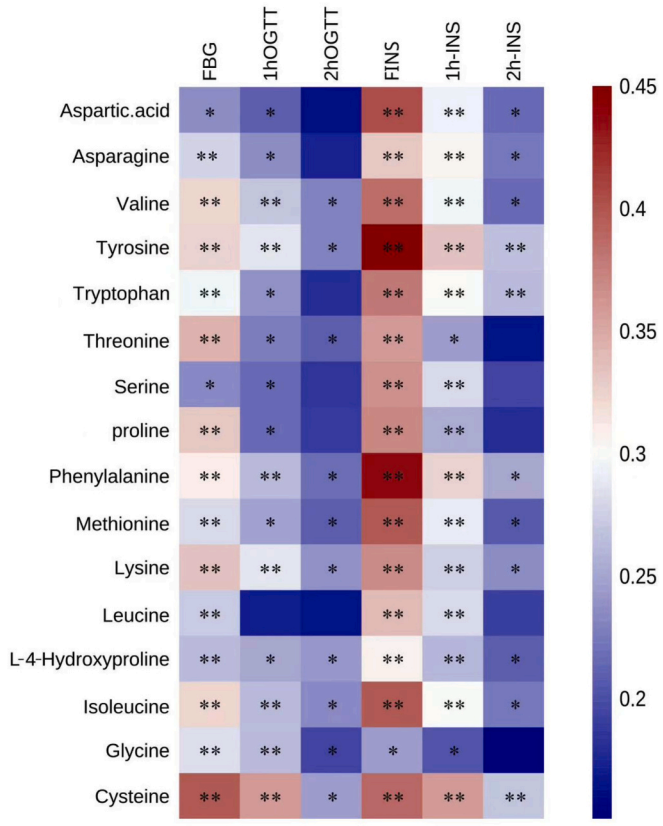

Fig. 1. Pearson correlations between amino acids when meet glucose and insulin metabolic parameters.
P values in bold indicate significant differences (* < 0.05 ** < 0.01, *** < 0.001). Adjusted for parity, Pre-pregnancy weight, age and weight at delivery.

3.4. Factors influencing insulin secretion and insulin resistance

We employed stepwise backward multiple linear regression analysis to explore baseline serum amino acid levels' impact on serum insulin levels at different time points and on insulin resistance indices. At fasting insulin levels, L-4-hydroxyproline, phenylalanine and serine showed effects, with phenylalanine being the sole positively correlated impact on fasting insulin secretion and producing the most potent effect (Table 5). Regression analyses of the R^2 values suggested these

Table 3
Plasma amino acid at 24–28 weeks of pregnancy.

Characteristics	Control group(n = 70)	GDM group(n = 35)	P value
Alanine(ng/ml)	13,416.29(8662.47,19,590.4)	17,589.67(13,894.28,22,097)	**0.048***
Asparagine(ng/ml)	3522.99(2102.13,7541.2)	8067.8(5399.71,9027.34)	**0.012***
Aspartic acid(ng/ml)	697.62(490.04,2265.54)	1922.87(1476.91,2574.02)	**0.002****
Cysteine(ng/ml)	652.11(455.43,3601.46)	3979.38(2500.59,4857.56)	**0.004****
Glutamic acid(ng/ml)	3038.7(2229.35,5120.15)	4063.43(3205.95016.9)	0.092
Glutamine(ng/ml)	97,637.9(46,081.97,139,163.04)	131,139.83(87,331.03,271,083.02)	0.150
Glycine(ng/ml)	824.47(589.98,2513.32)	2627.88(1492.81,3372.81)	**0.002****
Isoleucine(ng/ml)	1078.99(712.93,2513.64)	2431.57(1903.62968.16)	**0.002****
L-4-Hydroxyproline	316.99(211.76,1222.14)	1366.33(883.27,1758.56)	**0.001*****
Leucine(ng/ml)	13,371.08(10,304.16,18,600.53)	18,034.57(14,306.921918.01)	**0.014***
Lysine(ng/ml)	27,023.15(14,141.34,94,015.94)	87,798.06(65,938.59,100,269.25)	**0.014***
Methionine(ng/ml)	655.11(466.11,2349.87)	2042.49(1533.97,2399.41)	**0.005****
Phenylalanine(ng/ml)	4602.41(3089.918345.12)	17,850.45(13,314.322996.82)	**0.001*****
proline(ng/ml)	1823.1(1183.16,4014.13)	3847(2725,4706.64)	**0.009****
Serine(ng/ml)	2898.4(1900.56,9498.9)	8336.74(6985.33,10,131.67)	**0.015***
Threonine(ng/ml)	5417.48(3316.83,10,563.31)	10,926.15(7929.88,12,510.91)	**0.003****
Tryptophan(ng/ml)	43,431.01(26,468.88,151,404.28)	142,451.47(70,095.26,172,294.96)	**0.023***
Tyrosine (ng/ml)	2365.33(1530.89,11,280.94)	10,888.61(7970.23,13,790.83)	**0.004****
Valine (ng/ml)	4237.72(2842.18,11,492.25)	10,519.87(8544.97,13,309.66)	**0.002****

Data are presented as mean ± SD. P values in bold indicate significant differences(* < 0.05 ** < 0.01, *** < 0.001).

Table 4
Pearson correlations between BCAAs and AAAs when meet insulin metabolic parameters.

	Isoleucine	Leucine	Valine	Phenylalanine	Tyrosine	Tryptophan
FBG	0.261**	0.190	0.262**	0.255*	0.261**	0.234*
1hOGTT	0.236*	0.126	0.233*	0.236*	0.252*	0.193
2hOGTT	0.234*	0.174	0.226*	0.224*	0.22*	0.166
FINS	0.566***	0.516***	0.549***	0.606***	0.571***	0.466***
1 h-INS	0.352***	0.301**	0.341***	0.396***	0.394***	0.371***
2 h-INS	0.433***	0.425***	0.424***	0.473***	0.452***	0.419***
HOMA-IR	0.545***	0.491***	0.527***	0.581***	0.550***	0.448***

P values in bold indicate significant differences. (* < 0.05 ** < 0.01, *** < 0.001); Adjusted* for parity, Pre-pregnancy weight, age and weight at delivery;

Table 5
Parameters explaining the variation in insulin secretion and HOMA-IR in pregnant females on the basis of backward stepwise regression.

	β	P value
FINS $R^2 = 0.517$		
L-4-Hydroxyproline	−0.425	**0.021***
Phenylalanine	1.632	**0.001*****
Serine	−0.589	**0.030***
1 h-INS $R^2 = 0.344$		
Cysteine	0.863	**0.002****
Lysine	−0.572	0.057
Phenylalanine	0.858	**0.009****
Serine	−0.614	**0.047***
2 h-INS $R^2 = 0.409$		
Age	0.179	**0.034***
Asparagine	0.638	**0.046***
Aspartic acid	0.583	0.096
Cysteine	0.652	**0.023***
Phenylalanine	1.042	**0.040***
proline	0.481	**0.044***
Threonine	−0.687	**0.03***
HOMA-IR $R^2 = 0.551$		
Cysteine	0.644	**0.011***
Methionine	−1.158	**0.021***
Phenylalanine	1.465	**0.001*****
Serine	−1.071	**0.001*****
Tryptophan	−0.965	**0.001****
Tyrosine	0.985	**0.048***

Analysis; Standardized β-coefficients and P values are given; Skewed variables were logarithmically transformed before Included in the analysis (* < 0.05 ** < 0.01, *** < 0.001).

parameters accounted for 51% of the fasting insulin secretion variance. However, at 1-h insulin levels, the regression model yielded a decreased predictive power ($R^2 = 0.344$) with more subtle effects from cysteine, serine, and phenylalanine. In terms of 2-h insulin secretion levels, multiple influences were present, including the positive correlations of aspartic acid, asparagine, cysteine, phenylalanine, and proline, and the negative correlation of threonine. Adding to the amino acids, age also positively influenced the 2-h insulin levels. Concerning the final HOMA-IR metrics analysis, phenylalanine, cysteine, and tyrosine exhibited a positive effect on insulin resistance, whereas methionine, serine, and tyrosine had the opposite effect.

4. Discussion

Our study initially discovered that unusually high concentrations of specific amino acids served as independent risk factors for amplified insulin secretion, with the influence of phenylalanine on insulin resistance proving to be exceedingly noteworthy. Furthermore, phenylalanine exhibited a positive impact on the construction of linear regression models determining insulin secretion at varying time points. Aromatic amino acids and amino acids were positively correlated with blood glucose levels and serum insulin secretion levels at different times As a compensatory response to the abnormally escalated blood glucose levels, insulin secretion was markedly elevated at alternating time points in patients suffering from gestational diabetes mellitus (GDM), significantly exceeding the normal range. Recent research has investigated the correlation between branched-chain amino acids and the onset of Gestational Diabetes Mellitus (GDM) [22]; however, fewer studies have explored the metabolism of aromatic amino acids during pregnancy, resulting in inconsistent findings. For instance, serum sample analysis from early pregnancy (12–16 weeks of gestation) in Jiang's study [23] revealed significant differences in tyrosine levels between GDM patients and non GDM pregnancies (OR = 1.46). Similarly, Roy et al. [24] discovered significant disparities in levels of phenylalanine between those who developed GDM and the non GDM pregnancies among samples from 100 gestating women (7–15 weeks). However, the concentration of aromatic amino acids in late pregnancy remains a contentious issue. A study in Spain suggested elevated levels in late-term GDM patients [25], whereas a U.S study found no such discrepancies in peripheral serum phenylalanine levels between GDM patients and non GDM pregnancies [26]. This inconsistency might relate to increased demands for energy due to fetal growth and enhanced placental transport of amino acids in late pregnancy [27]. Recent studies have, nonetheless, pointed to abnormally high levels of aromatic amino acids in GDM patients in mid-pregnancy, these differences have been consistently noted at various stages of the mid-pregnancy OGTT experiment [19]. Our data from quantitative amino acids testing at fasting level aligns with these findings, displaying elevated levels of multiple amino acids, principally AAAs and BCAAs.

Our study noted correlations between several amino acids and both insulin resistance and secretion, with branched-chain and aromatic amino acids being particularly pronounced. Aromatic amino acids exhibited a slightly stronger correlation with insulin secretion and resistance than branched-chain amino acids - a nuance that varies slightly from other similar studies [24–26]. In further multiple linear regression analyses including birth order, age, BMI at sampling, and different amino acid metrics to assess their impact on insulin-related metrics, we found that phenylalanine was a common factor across all evaluated metrics. The strongest correlations were with fasting insulin levels and insulin resistance metrics, followed by an effect on insulin levels at 2 h post-OGTT, with the least significant impact on 1-h insulin levels. While the correlation between baseline serum amino acid levels and fasting amino acid indexes is robust, the role of phenylalanine as a marker of insulin levels, particularly after insulin level disturbances driven by food ingestion, should not be downplayed.

Recognizing the differential mechanisms between fasting insulin secretion and post-fasting insulin secretion and the association of phenylalanine with insulin indicators at varied times [28], we speculate that phenylalanine's role in inducing compensatory insulin secretion and resistance relates to the downstream effects of insulin action. How phenylalanine impacts insulin signaling in Gestational Diabetes Mellitus (GDM) remains uncertain; yet, insulin action mechanisms are better understood in the context of both normal individuals and Type 2 Diabetes Mellitus (T2DM). In normal individuals, orally absorbed phenylalanine can directly stimulate insulin secretion via the Calcium-Sensing

Receptor(CaSR)[29,30] and stimulate Glucose-dependent Insulinotropic Polypeptide (GIP) secretion to elevate serum insulin levels.[31] However, the stimulation of insulin secretion varies between baseline elevations of phenylalanine that result in increased insulin levels and brief surges of phenylalanine. We hypothesize that phenylalanine introduces insulin resistance via a novel mechanism of action. In T2DM, deviant glucose-lipid metabolism provokes oxidative stress and inflammation, leading to insulin signaling disruption when isomers of tyrosine derived from phenylalanine are incorporated into the IRS-1 receptor [32]. Adverse amounts of phenylalanine integrated with IR-β cause a loss of tyrosine protein kinase attributes [33], thereby inhibiting the subsequent downstream insulin signaling pathway. Phenylalanine has served as an established molecular marker with predictive value in T2DM. Given that GDM bears similarities to T2DM pathogenesis, especially with heightened oxidative stress during pregnancy [34,35], we posit that baseline phenylalanine levels may differentially impact insulin function. Consequently, the body may resort to secreting higher insulin levels to compensate for decreased insulin bioavailability during pregnancy phases, intensifying the islet burden and the potential progression risk to T2DM [36].

Our study discovered for the first time how fasting phenylalanine levels consistently interfere in multiple insulin-related markers in GDM, thereby contributing to explaining the abnormal shifts in insulin resistance and secretion. Given that most research[22,23] indicates abnormally elevated phenylalanine levels early in pregnancy among those diagnosed with GDM, phenylalanine has potential as an indicator for assessing GDM risk and insulin secretion levels in affected pregnant women.

However, this study does have limitations. While the analyses manifest that phenylalanine impacts insulin secretion and resistance indices, no specific mechanistic studies in GDM exist to demonstrate phenylalanine's direct effect on insulin signaling during pregnancy. Compared to models using fasting insulin resistance indices and insulin secretion levels, the capability of these models to depict 1 h and 2 h insulin secretion levels was relatively weak, indicating a need for more objective evaluation of the influence of phenylalanine levels at the same time point on insulin secretion. As a study focusing on the serum amino acid levels during mid-pregnancy, its predictive power is limited, suggesting a need for earlier testing to achieve optimal preventive effects. In addition, we matched weight and excluded corresponding obese individuals in the process of including GDM patients and non-GDM population. This description may be more applicable to the population in Asia, especially the Chinese population. Compared with Asian pregnant women, pregnant women from other countries have a significantly increased risk of obesity.

Despite the limitations, we discerned phenylalanine's independent influences on insulin secretion and resistance at varying time points in GDM patients. These findings offer valuable insights into the pathogenesis of GDM, further presenting strategic values on mitigating hyper stimulation of insulin secretion and preserving residual pancreatic islet functionality, critical for subsequent preventative measures aimed at curtailing the progression to Type 2 Diabetes Mellitus (T2DM). Future inquiry into the mechanistic studies remains necessary, and a more comprehensive investigation is required to definitively establish the direct relationship between phenylalanine and insulin secretion.

Author contributions

Prof. Danqing Chen and Yanmin Chen designed the experiments and revised the article, Hao Wu was responsible for the experiments and data compilation and analysis, and all authors approved the final manuscript.

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CRediT authorship contribution statement

Hao Wu: Conceptualization, Methodology, Software, Investigation, Formal analysis, Writing – original draft, Visualization, Writing – review & editing. **Qiong Wang:** Data curation, Writing – original draft. **Yanmin Chen:** Visualization, Investigation, Resources, Supervision. **Danqing Chen:** Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

There is no dispute of mutual financial interests.

Data availability

Data will be made available on request.

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