Original Article

Association between dietary protein intake and preterm birth in pregnant women with gestational diabetes mellitus: the WeBirth cohort study

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Background and Objectives: The distribution of dietary macronutrients is essential for blood glucose management in patients with gestational diabetes mellitus (GDM). However, the relationship between dietary protein intake and the risk of preterm birth remains unclear. Here, we aim to investigate the prospective association between dietary protein intake and preterm birth in patients with GDM. **Methods and Study Design:** We included 1756 GDM patients and assessed dietary protein patterns by constructing total protein index (TPI), animal protein index (API), and plant protein index (PPI) using data collected from food frequency questionnaires (FFQ). **Results:** We found that individuals in the highest quartile of TPI (OR: 2.75, 95% CI: 0.81 to 9.22) and API (OR: 3.64, 95% CI: 1.48 to 9.47) had a significantly higher risk of preterm birth compared to those in the lowest quartile. **Conclusions:** This study suggests that increasing protein intake, especially from animal sources, was associated with a greater risk of preterm birth in patients with GDM.

Key Words: preterm birth, gestational diabetes mellitus (GDM), protein intake, cohort, FFQ

INTRODUCTION

Gestational diabetes mellitus (GDM) is a type of diabetes that occurs during pregnancy and affects approximately 14% of pregnant women worldwide.[1, 2] Women with GDM have a higher risk of preterm birth and other pregnancy complications compared to those with normal blood glucose levels during pregnancy.[3] Preterm birth, defined as delivery after 28 weeks but before 37 weeks of gestation, is a leading cause of neonatal death and long-term health problems globally.[4] Statistics indicate that the global preterm birth rate is about 11%, resulting in approximately 15 million premature births annually.[5] Preterm birth has serious implications for both offspring and maternal health, including increased risks of neonatal respiratory distress syndrome, cerebral palsy, developmental delay in offspring,[6] and postpartum depression and chronic diseases in mothers.[7] Therefore, discovering modifiable factors that reduce the risk of preterm birth complicated by GDM was crucial.

In recent years, nutritional status during pregnancy, particularly protein intake, has emerged as a key factor influencing pregnancy outcomes. Protein is essential for fetal growth and development, as well as placenta formation, fetal organ development, and maternal metabolic regulation.[8] Research has shown that both insufficient and excessive protein intake during pregnancy may have unfavorable influence of fetal growth.[9] Furthermore, the impact of dietary protein intake patterns, such as diets rich in plant or animal protein, on pregnancy outcomes is of significant concern. Diets high in plant protein may reduce the risk of preterm birth due to their anti-inflammatory and antioxidant effects,10 whereas diets high in animal protein may be associated with increased levels of inflammatory markers.[11] However, most existing studies focus on individual nutrients or food groups, lacking a comprehensive assessment of overall protein intake patterns. Dietary pattern analysis, compared to single-nutrient studies, can more comprehensively reflect the

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complexity of dietary structures.[12]

GDM has unique pathophysiological characteristics compared to uncomplicated gestations and findings from uncomplicated pregnancies may not be applicable to those with GDM.[13] Therefore, specific studies are necessary to elucidate the association between dietary protein intake during pregnancy and the risk of preterm birth in women with GDM. However, this association remains unclear. To this end, we investigate the relationship between dietary protein intake patterns during pregnancy and preterm birth among GDM patients.

METHODS

Study population

The study was based on data from the Westlake Precision Birth Cohort (WeBirth) in Hangzhou, China. All study participants provided written informed consent and the study was approved by the Institutional Review Board of Westlake University, Hangzhou, China (20190701ZJS0007). WeBirth was an ongoing prospective cohort study enrolling pregnant women with GDM from the Hangzhou Maternal and Child Health Hospital in Hangzhou, China, starting in August 2019.[14] Inclusion criteria included: (1) being 18 years of age or older, having been diagnosed with GDM, and being between 24 and 28 weeks of gestation; (2) Pregnant women who intend to give birth in Hangzhou Women's and Children's Hospital and live in Hangzhou with their children for 4 years or more. Pregnant women with cancer or infectious diseases, who tested positive for the three major markers of hepatitis B (hepatitis B surface antigen, hepatitis B core antigen, and Hepatitis B e antigen), syphilis antibodies, or HIV infection were excluded from the study. The diagnosis of gestational diabetes was based on the criteria of the International Association of Diabetes and Pregnancy Research Groups, which encompasses fasting plasma glucose greater than or equal to 91.9 mg/dL (to convert to millimoles per liter, multiply by 0.0555), and/or 1 hour plasma glucose greater than or equal to 180.0 mg/dL, and/or 2-hour plasma glucose greater than or equal to 153.3 mg/dL.[15]

As of August 2023, the WeBirth cohort has enrolled 2,001 participants. In this analysis, we excluded participants with the following conditions: 1) those who had no Food Frequency Questionnaire (FFQ) records (n = 22); 2) those who did not deliver in the present hospital (n=192); 3) who did not provide baseline demographic information, such as pre-pregnancy body mass index(pre-pregnancy BMI), education, income, smoking or drinking status (n = 31). The final sample size was 1756 in the present analysis.

This study was reviewed and approved by the Ethics Committee of Westlake University and the Ethics Committee of Hangzhou Obstetrics and Gynecology Hospital, with the approval numbers being 20190701ZJS0007 and [2022] Medical Ethics Review A No. (3) -01 respectively, and each participant obtained a written informed consent form.

Dietary assessment and covariate collection

During the recruitment process, we used a validated FFQ to collect information about the participants' dietary habits over the past month. Questionnaires filled out by the trained interviewers were used to gather information about demographics, lifestyle, medical history, and health conditions before and during pregnancy. Information on participants' physical activity was collected using the Pregnancy Physical Activity Questionnaire (PPAQ).[16] Other covariates were also collected at baseline using questionnaires, including age, pre-pregnancy BMI, gestational week, parity, income, smoking and alcohol consumption status, and family history of diabetes.

Calculation of dietary protein intake index

Based on FFQ data, we constructed three protein intake scores, including total protein index (TPI), animal protein index (API), and plant protein index (PPI). Daily intake of total protein, animal protein, and plant protein (unit: g/day) was calculated for each participant by taking account into the food types, consumption frequencies, and portion sizes derived from the FFQ. Specifically, total protein intake was defined as the sum of animal and plant protein intake. For each protein category (total, animal, and plant), all participants were ranked by ascending daily intake values. The cohort was then stratified into four quartile groups based on these rankings: the first quartile (Q1, representing the lowest 25% of intake) was assigned a score of 1, the second quartile (Q2, 25%–50%) a score of 2, the third quartile (Q3, 50%–75%) a score of 3, and the fourth quartile (Q4, highest 25%) a score of 4. The TPI, API and PPI for each participant were determined by their respective quartile-based scores (1-4) across the three protein categories. Higher scores indicate a higher relative intake level of the corresponding protein type within the study population.

Pregnancy outcome

The pregnancy outcome of interest in the present analysis was the delivery of preterm fetus. During the delivery, we obtained the gestational week of the participants from their medical records. According to the World Health Organization,17 preterm birth was defined as babies born alive before 37 weeks of pregnancy.

Statistical analysis

All statistical analyses were performed in Stata 16.0 (Stata Corporation, College Station, TX, USA) or R (version 4.2.2). The baseline population characteristics were presented as the mean (standard deviation) for continuous variables and n (%) for category variables. The protein index was divided into four groups based on the quartiles. this approach was taken to minimize potential recall errors associated with FFQ-based protein calculations.

Logistic regression models

For the primary analysis, logistic regression analysis was performed to estimate the association of TPI, API, and PPI with preterm birth. We fitted two statistical models, with model 1 adjusting for age, gestational week at data collection, pre-pregnancy BMI and parity, and model 2 further adjusting for education level, income, smoking and alcohol consumption. As a secondary analysis, we analyzed the association of API and PPI with preterm birth after controlling for total protein intake. We fitted two statistical models again, with model 1 adjusting for

age, gestational week at data collection, pre-pregnancy BMI, total energy intake, parity, and total protein intake, and model 2 further adjusting for education level, income, smoking and alcohol consumption status and total protein intake. In this study, Benjamini-Hochberg method was used to correct multiple tests, and FDR<0.05 was considered statistically significant.

Sensitivity analysis

Since participants' lipids, blood pressure, and hemoglobin may influence the relationship between dietary protein intake and preterm birth, we further corrected for these indices. Specifically, we further adjusted for systolic blood pressure (SBP) in model 2a, low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG) in model 2b, and hemoglobin a1c (HbA1c) in model 2c. In these sensitivity analyses, we excluded participants with missing values (lipid profile n=206, blood pressure n=3, HbA1c n=120.

RESULTS

Characteristics of study participants

We included 1756 participants in this analysis, with the age ranging from 20.8 to 44.0 years (mean age, 32.5; SD, 4.6). More than half of these women (66.7%) were primiparous. The average pre-pregnancy BMI (SD) of the included participants was 22.2 (3.5) kg/m^2. Among the 1756 individuals, 64 were preterm births, resulting in a preterm to term birth ratio of 64:1692 in the overall population. When stratified by Total Protein-based Q1-Q4 subgroups, the preterm to term birth ratios were 11/428, 10/429, 17/422, and 26/413, respectively. Women with higher overall protein index scores were more likely to be primiparous and had higher annual incomes (Table 1).

Association between dietary protein patterns and preterm birth

The result of our primary analysis showed that participants in the top quartile of the TPI had a higher risk of preterm birth compared to the bottom quartile (OR: 2.75, 95% CI: 0.81 to 9.22, FDR<0.02). We also found similar results which showed that participants in the top quartile of the API were more likely to have a higher risk of preterm birth (OR: 3.61, 95% CI: 1.48 to 9.47, FDR<0.01) (Table 2). We did not find any associations between increased PPI and preterm birth. Our secondary analysis showed no significant associations of API or PPI with preterm birth after controlling for TPI (Table 3). Our sensitivity analyses revealed consistent associations of TPI and API with the risk of preterm birth (Table 4).

DISCUSSION

Leveraging dietary data obtained from FFQ in a large cohort of GDM patients, we differentiated TPI, API, and PPI to analyze their individual impact on the risk of preterm birth. Notably, this study chose to conduct dietary assessment during the second trimester of pregnancy. During this period, compared with the first trimester, nausea symptoms are alleviated, and gastrointestinal compression is less severe than in the third trimester, which reduces individual dietary variability and significantly improves the accuracy of FFQ data, providing an important guarantee for the reliability of the research results. The results of our study indicated that higher overall protein intake, particularly API was significantly associated with an increased risk of preterm birth. In contrast, no significant association was observed between PPI and the

Table 1. Population characteristics by quartiles of total protein intake index

	Total protein			
	Q1 (N = 356)	Q2 (N = 496)	Q3 (N = 431)	Q4 (N = 473)
Age, years	31.4 (3.7)	31.2 (3.9)	31.5 (3.6)	31.1 (3.6)
pre-pregnancy BMI, kg/m^2	22.1 (3.2)	22 (3.1)	21.9 (3.2)	21.9 (3)
Gestational week, weeks	26 (1.9)	25.9 (1.8)	25.9 (1.9)	25.9 (1.6)
Primiparity	268/439 (61.0)	292/439 (66.5)	289/439 (65.8)	312/439 (71.1)
Educational level				
<High school or vocational school	76/439 (17.3)	42/439 (9.6)	38/439 (8.7)	47/439 (10.7)
University or professional school	311/439 (70.8)	326/439 (74.3)	329/439 (74.9)	333/439 (75.9)
>University	52/439 (11.8)	71/439 (16.2)	72/439 (16.4)	59/439 (13.4)
Household income RMB/year				
<100,000	111/439 (25.3)	96/439 (21.9)	85/439 (19.4)	92/439 (21.0)
100,000-200,000	159/439 (36.2)	172/439 (39.2)	144/439 (32.8)	163/439 (37.1)
>200,000	169/439 (38.5)	171/439 (39.0)	210/439 (47.8)	184/439 (41.9)
Smoking				
Never	424/439 (96.6)	424/439 (96.6)	417/439 (95.0)	418/439 (95.2)
Ever	15/439 (3.4)	15/439 (3.4)	22/439 (5.0)	20/439 (4.6)
Alcohol drinking				
Never or seldom	289/439 (65.8)	315/439 (71.8)	294/439 (67.0)	273/439 (62.2)
Occationally	150/439 (34.2)	124/439 (28.2)	145/439 (33.0)	166/439 (37.8)
LDLC, mmol/L	3.1 (0.7)	3.1 (0.7)	3.1 (0.7)	3.1 (0.7)
TG, mmol/L	2.5 (1)	2.5 (1.1)	2.5 (0.9)	2.5 (0.9)
SBP, mmHg	112.6 (11.8)	113.7 (12.5)	113.5 (11.3)	115.1 (11.3)
HbA1c,mmol/mol	5 (0.3)	5 (0.3)	5 (0.3)	5 (0.4)

Q, quartile; BMI, body mass index; MET, metabolic equivalent; Total number of participants: 1,756.
Data were presented as mean (standard deviation) for continuous measures, and n (%) for categorical measures.

Table 2. Associations between protein intake and preterm

	Total protein		Animal protein		Plant protein	
	ORs (95%CI)	FDR	ORs (95%CI)	FDR	ORs (95%CI)	FDR
Preterm						
Q2 vs. Q1	0.93 (0.37, 2.34)	0.88	1.15 (0.47, 2.83)	0.88	0.93 (0.42, 2.05)	0.88
Q3 vs. Q1	1.66 (0.67, 4.24)	0.61	1.26 (0.51, 3.18)	0.61	0.71 (0.31, 1.70)	0.61
Q4 vs. Q1	2.75 (0.81, 9.22)	0.03	3.64 (1.48, 9.47)	0.02	0.43 (0.14, 1.35)	0.15
p for trend	<0.02		<0.01		0.16	

Q, quartile; ORs, odds ratio; CI, Confidence interval; FDR, error discovery rate
OR (95% CI) was derived from a multivariate adjusted logic model of the protein intake index. Covariates included age, Gestational week, pre-pregnancy BMI, total energy intake, parity, and total protein intake, education level, income, smoking and alcohol consumption status.

Table 3. Relationship between animal and plant protein and preterm birth after controlling for total protein

	Animal protein		Plant protein	
	ORs (95%CI)	FDR	ORs (95%CI)	FDR
Preterm				
Q2 vs. Q1	1.20 (0.46, 3.13)	0.77	0.89 (0.41, 1.97)	0.77
Q3 vs. Q1	1.40 (0.44, 4.52)	0.57	0.65 (0.28, 1.54)	0.57
Q4 vs. Q1	4.20 (1.09, 17.55)	0.09	0.42 (0.14, 1.28)	0.13
p for trend	0.05		0.11	

Q, quartile; ORs, odds ratio; CI, Confidence interval; FDR, error discovery rate.
OR (95% CI) was derived from a multivariate adjusted logic model of the protein intake index. Covariates included age, gestational week, pre-pregnancy BMI, total energy intake, parity, and total protein intake, education level, income, smoking and alcohol consumption status, and total protein intake.

Table 4. Associations between protein intake and preterm

	Total protein		Animal protein		Plant protein	
	ORs (95%CI)	FDR	ORs (95%CI)	FDR	ORs (95%CI)	FDR
Preterm						
Model 2	1.44 (0.96,2.14)	0.11	1.56 (1.15, 2.13)	0.02	0.77 (0.54, 1.10)	0.16
Model 2a	1.44 (0.97, 2.15)	0.11	1.67 (1.07, 2.66)	0.08	0.75 (0.53, 1.07)	0.11
Model 2b	1.93 (1.23, 3.04)	0.01	2.3 (1.34, 4.06)	0.01	0.65 (0.44, 0.96)	0.03
Model 2c	1.67 (1.1, 2.56)	0.05	1.7 (1.06, 2.81)	0.05	0.76 (0.53, 1.1)	0.15

CI, Confidence interval; SBP, systolic blood pressure; HbA1c,hemoglobin a1c;LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; ORs, odds ratio; FDR, error discovery rate.
OR (95% CI) was derived from a multivariate adjusted logic model of the protein intake index. Covariates included age, gestational week, pre-pregnancy BMI, total energy intake, parity, and total protein intake, education level, income, smoking and alcohol consumption status, total protein intake, SBP ,LDL-C ,TG and HbA1c .Model 2a further adjusts systolic blood pressure (SBP) on the basis of model 2, model 2b further adjusts low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG), and model 2c further adjusts hemoglobin a1c (HbA1c).

risk of preterm birth. These findings suggested that the source of protein may play a crucial role in the occurrence of preterm birth, rather than simply the TPI.

The results of our study were generally consistent with some existing studies. For example, Martin et al. found that high dairy intake (rich in animal protein) was associated with an increased risk of preterm birth.[18] Similarly, Lu et al. found in a prospective cohort study of pregnant women in China that women with higher dairy intake had a higher risk of preterm birth, further supporting the findings of our study.[19] However, Alves-Santos et al. came to a different conclusion, finding that high dairy intake during pregnancy was associated with a reduced risk of preterm birth.[20] Such differences may be due to differences in study design, sample characteristics, or dietary assessment methods. In addition, the study by Grieger et al. found that high protein intake was associated with a reduced risk of preterm birth, but this study did not distinguish between animal and plant protein, which may explain part of the difference with our findings.[21]

Our study did not find a significant association between plant protein intake and the risk of preterm birth, which was consistent with the findings of several studies. For example, Haugen M et al. found no association between a Mediterranean-style diet (predominantly plant foods and low meat consumption) and preterm birth in a study of a Norwegian cohort of pregnant women,[22] and another retrospective study of women in the French Caribbean Mother and Child Cohort Study (TIMOUN) showed that adherence to a Mediterranean-style diet was associated with a reduced risk of preterm birth.[23] However, the findings only apply to overweight and obese women.

Higher overall protein intake, especially animal protein intake, was associated with an increased risk of preterm birth. This result may be related to the metabolic characteristics of animal protein and its influence on physiological state during pregnancy. First, animal protein was often rich in saturated fatty acids and cholesterol, which may affect placental function by increasing levels of

inflammatory response and oxidative stress.[24] Studies had shown that high animal protein intake was associated with increased serum levels of inflammatory markers such as C-reactive protein and interleukin-6, and that inflammatory responses play a key role in the development of preterm birth.[25] Second, animal protein intake may increase the risk of preterm birth by affecting hemorheological properties. Yip et al. showed that high animal protein intake may lead to increased hemoglobin concentration,[26] which increased blood viscosity. Excessive blood viscosity may disrupt the microcirculatory system, affect placental blood perfusion, and lead to fetal stress and premature delivery. The placenta was the only organ that connects the mother and the fetus, and poor blood perfusion in the placenta can directly affect the nutrient supply and oxygen exchange of the fetus, increasing the risk of premature birth. In contrast, plant protein intake was not significantly associated with the risk of preterm birth, which may be related to the anti-inflammatory and antioxidant properties of plant protein. Plant protein sources were often rich in dietary fiber, polyphenols, and other bioactive substances that may help maintain inflammatory balance and REDOX homeostasis during pregnancy. For example, isoflavones in soy protein had anti-inflammatory and antioxidant effects and may have a positive impact on pregnancy outcomes.[27] In addition, plant protein intake was often associated with healthier eating patterns, such as a high-fiber, low-saturated fat which may further reduce the risk of preterm birth.

The results of our research were of great theoretical and practical significance. The findings supported the important role of protein sources in maintaining healthy pregnancy, providing a novel perspective on GDM patient care through the modulation of dietary macronutrient distributions. In particular, the relationship between API and inflammatory response, hemorheological properties, and placental function provided directions for future mechanism studies. In practice, the findings suggested that clinicians and dietitians should consider the importance of protein sources when formulating dietary recommendations for pregnant women. For example, advising pregnant women to reduce their intake of animal protein, especially animal foods high in saturated fat (such as red meat and full-fat dairy products), while increasing their intake of plant protein (such as legumes, nuts, and whole grains), may help reduce the risk of preterm birth. In addition, public health policymakers should consider emphasizing the importance of protein sources in nutrition guidelines during pregnancy and providing specific dietary recommendations to help pregnant women optimize their diets.

Although our study provided valuable insights, there were some limitations. First, the dietary data relied on self-reporting by pregnant women, with possible recall bias and reporting errors. Although we used the validated FFQ, we could not completely rule out the effect of measurement error. In addition, although we adjusted for multiple confounders, there may still be unmeasured confounders that influence the results. Future studies should use more comprehensive data collection methods to control for potential confounding factors. In terms of sample selection, our study only targeted pregnant women with GDM, which may limit the generality of the results.

Conclusion

Our study provided new evidence for both academic advancement and practical application in this field by exploring the association between dietary protein intake patterns and the risk of preterm birth. Future studies should further validate these findings and explored potential interventions to improve pregnancy outcomes.

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CONFLICT OF INTEREST AND FUNDING DISCLOSURES

The authors declare no conflict of interest.

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