Study on the Correlation Between Homocysteine-related Dietary Patterns and Gestational Diabetes Mellitus Based on Reduced-rank Regression Analysis

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Research Article

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Abstract

**Background:** This study aimed to evaluate the association between homocysteine-related dietary patterns and gestational diabetes mellitus.

**Methods:** A total of 488 pregnant women at 24–28 weeks of gestation between January 2019 and December 2020 were included. Demographic characteristics, dietary intake, and multivitamin supplement intake information were collected using a food frequency questionnaire (FFQ); fasting venous blood samples were collected for serum index detection. Serum homocysteine (Hcy), folic acid, and B12 were selected as response variables, and hyperhomocysteinemia (hHcy)-related dietary patterns were extracted using the descending rank regression method. The relationship between the score of hHcy-related dietary patterns and GDM was analyzed using a multivariate logistic regression model.

**Results:** Three hHcy-related dietary patterns were extracted. Only mode 2 had a positive and significant relationship with the risk of developing GDM. After adjusting for confounding factors, the risk of GDM was significantly increased in the highest quartile array compared with the lowest quartile of the pattern (OR=2.96, 95% Confidence Interval: 0.939–9.356, \(P=0.004\)). There was no significant correlation between dietary pattern 1 and GDM risk (\(P>0.05\)).

**Conclusions:** Homocysteine-related dietary patterns were positively associated with gestational diabetes mellitus. Adjusting dietary patterns may contribute to the intervention and prevention of GDM.

Background

Due to the increase in the incidence of obesity and elderly parturients, the incidence of gestational diabetes mellitus (GDM) in China has been increasing by 14.8% annually with regional variation, wherein East China, Central China, and North China have a higher prevalence [1]. The prognosis of GDM mainly depends on early prevention and intervention, among which dietary therapy is an important strategy for its primary prevention and is the basis of all diabetes treatments [2]. Dietary pattern is a comprehensive evaluation of diet as a whole, which can accurately reflect the effect of diet on diseases [3] and is significant in the study of the relationship between nutrition and health [4]. Studies have found that high homocysteine levels (hyperhomocysteinemia, hHcy) are risk factors for GDM [5], and dietary patterns affect serum homocysteine (Hcy) levels; for example, a Mediterranean diet [6] and frugal diet [7] can significantly reduce serum Hcy levels. However, whether dietary patterns affect the incidence of GDM through changes in Hcy levels and its related mechanisms remains unelucidated.

There are three types of dietary pattern-extraction methods: first is the use of dietary indices (DI); second is induction, which includes principal component analysis (PCA), cluster analysis (CA), and factor analysis (FA); and third is the combination of methods from the first and second classes, which includes reduced rank regression (RRR) and partial least-squares regression (PLS). Adopting different methods according to the research purpose, the RRR method can explain the reaction variables to the greatest extent, such as variables related to disease outcomes and nutrients, explain the variations [8], contribute to the analysis of the relationship between dietary patterns and disease, and analyze its possible mechanism. The applications of RRR are expanding in the field of nutrition epidemiology [9]. Therefore, this study aimed to extract hHcy-related dietary patterns using the RRR method and analyze their relationship with the incidence of GDM to explore the possible role of dietary patterns in Hcy levels and GDM.

Methods

Subjects

A total of 512 pregnant women at 24–28 weeks of gestation who underwent regular obstetric examinations at our hospital between January 2019 and December 2020 were included in the study. Inclusion criteria were single pregnancy,
normal expression and understanding ability, and informed consent. Exclusion criteria were: history of diabetes mellitus, hypertension, and thyroid disease; acute or chronic infectious diseases with obvious symptoms; and other major diseases. This study was approved by the Medical Ethics Committee of our hospital. All study participants provided informed consent. This study was approved by the Medical Ethics Committee of the Seventh People's Hospital Affiliated to Shanghai University of Chinese Medicine (ethics batch number: 2019-7th-HIRB-014). All the study participants provided informed consent.

**Research Methods**

**General demographic characteristics:** Sociodemographic data (age, education, and gestational age) and pregnancy history (number of pregnancies/births) were collected by trained investigators. Height and weight were measured using uniform standards and specifications, and body mass index (BMI) before pregnancy was calculated to record weight gain during pregnancy.

**Dietary questionnaire survey:** Dietary review method and food model were used to collect dietary intake and multivitamin supplement intake of pregnant women during pregnancy through face-to-face interviews with Food Frequency Questionnaire (FFQ). At the same time, pregnant women were asked about their intake of nutritional supplements, and their dietary intake of folic acid, vitamin B6 and vitamin B12 was recorded according to the product instructions of nutritional supplements. According to the food classification principles in the Chinese Food Composition List (sixth edition) [10], food types are classified and sorted into 24 types of food groups. All food intake data were standardized using NutritionStar software (Yingkang Technology Company). The specific method was as follows: the intake of each food group was equal to each intake multiplied by the daily intake times. The daily intake times were converted from the intake frequency. Food with an intake proportion ≤ 5% (crab/shell, egg tarts/shaomai, coffee/tea, and condiments) were not included in the dietary pattern analysis. In addition, subjects whose intake frequency of the 24 food groups was > 99% with an energy intake of < 800 kcal were excluded. Finally, 488 cases were included in the analysis.

**Diagnostic criteria for gestational diabetes:** Pregnant women were screened for gestational diabetes mellitus at 24–28 weeks of gestation (referred to as "glucose screening"). Glucose screening was a 75 g oral glucose tolerance test (OGTT) according to China's Guidelines for the Diagnosis and Treatment of Gestational Diabetes Mellitus (2014) [11]. GDM is diagnosed if the blood glucose level reaches or exceeds the lower limit as follows: Fasting blood glucose (FBG) 5.1 mmol/L, 1-h postprandial blood glucose (1 h PG) 10.0 mmol/L, and 2-h postprandial blood glucose (2h PG) 8.5 mmol/L. According to the results from the OGTT, patients were divided into normal groups (n = 345) and GDM groups (n = 143).

**Serum index detection:** The blood glucose in the OGTT was measured using the hexokinase method with a Beckman automatic biochemical analyzer (AU5800). Serum Hcy was detected using the enzyme cycle method with a Beckman automatic biochemical analyzer (AU5811). Serum folic acid (FA) and B12 folic acid were determined by the chemiluminescence method using an Abbott Automatic Immunoanalyzer (I1000S). Quality control was performed for all tests prior to testing. When the quality was not controlled, the specific reasons were analyzed and dealt with accordingly. Finally, tests were performed after re-controlling.

**Statistical Methods**

Differences between the GDM and normal groups were compared using the t-test or χ² test. RRR analysis was performed with the option (METHOD = RRR) in the PLS process of SAS software version 9.4 (SAS Institute, North Carolina, USA). Serum Hcy, FA, and B12 values were used as response variables, and as the RRR method could obtain at most the same number of dietary patterns as the number of response variables, three dietary patterns explaining hHcy variation could be obtained in this study. The dietary pattern factor load represented the size and direction of each food group's contribution to hHcy-related dietary patterns, and the dietary pattern score was obtained by multiplying the dietary pattern factor load by the standardized food intake. The relationship between the scores of the three dietary patterns and the intake of each
food group was evaluated using Pearson's correlation. The subjects were divided into four groups according to the quartile of dietary pattern score, the characteristics of the subjects were analyzed, and a trend analysis was performed. The quartiles of dietary scores were used as independent variables, and logistic regression was used to analyze the relationship between hHcy-related dietary pattern scores and GDM after adjusting for age, educational background, gestational grade, BMI before pregnancy, weight gain during pregnancy, energy intake, and multivitamin intake.

Results

General Features

There was no difference in educational background and weight gain during pregnancy in the GDM group compared to the normal group (P < 0.01). However, patients in the GDM group were older, the proportion of postpartum women was higher, and the pre-pregnancy BMI and energy intake levels were higher (P < 0.01). The intake of folate in the GDM group was lower than that in the normal group, but there was no difference in the intake of B12 and B6 between the two groups (P > 0.01). Serum Hcy levels were higher in the GDM group, but FA and B12 levels were lower in the GDM group than in the normal group (P < 0.01) (Table 1).
Table 1  
Comparison of basic characteristics between the GDM and normal groups

| Essential characteristics | OGTT | t-value | P-value |
|---------------------------|------|---------|---------|
|                           | normal group (n = 345) | GDM group (n = 143) |
| Age                       | 28.513 ± 4.447 | 30.636 ± 4.645 | 4.739 | < 0.001 |
| Educational background    |                                | -0.576 | 0.565 |
| Senior high school and below | 188 (54.50) | 82 (57.34) |
| College degree or above   | 157 (45.50) | 61 (42.66) |
| Gravidity                 |                                | -2.475 | 0.013 |
| Primipara                 | 180 (52.17) | 57 (39.86) |
| Multipara                 | 165 (47.83) | 86 (60.14) |
| Pregnancy BMI (kg/m²)     | 22.097 ± 3.629 | 23.992 ± 4.527 | 4.450 | < 0.001 |
| Weight gain during pregnancy (kg) | 8.774 ± 4.144 | 8.794 ± 4.992 | 0.045 | 0.964 |
| Energy intake (kcal/d)    | 1464.085 ± 411.720 | 1642.900 ± 636.197 | 3.103 | 0.002 |
| Multivitamin supplement intake |                               |         |       |
| dietary FA (ug)           | 538.936 ± 572.832 | 431.846 ± 445.723 | 1.998 | 0.028 |
| dietary B12 (ug)          | 2.631 ± 6.828 | 2.472 ± 9.833 | 0.204 | 0.838 |
| dietary B6 (mg)           | 1.974 ± 2.288 | 1.772 ± 2.502 | 0.862 | 0.389 |
| Serological indicator     |                                |         |       |
| serum FA (nmol/L)         | 21.967 ± 10.523 | 18.189 ± 14.161 | 2.877 | 0.004 |
| serum B12 (pmol/L)        | 219.294 ± 110.621 | 188.632 ± 99.506 | 2.997 | 0.003 |
| serum Hcy (umol/L)        | 5.770 ± 1.668 | 7.235 ± 5.007 | 3.423 | < 0.001 |

Note: Categorical variables include educational background and pregnancies, expressed as the number of people (constituent ratio). Continuous variables included age, pre-pregnancy BMI, weight gain during pregnancy, energy intake, intake of multivitamin supplements (FA, B12, and B6), and serological indicators (FA, B12, and Hcy), expressed as mean ± standard deviation, in which energy intake did not include the energy provided by cooking oil intake.

**Characteristics of RRR dietary pattern**

Three dietary patterns were identified in the present study (Table 2). For mode 1, the correlation index was > 0.20 mainly for poultry meat and livestock meat; and < -0.20 for green leafy vegetables, dark vegetables, soybeans, and shrimp, which explained the 29.14% variation in food and 24.26% variation in response variables. For mode 2, the correlation index was > 0.20 mainly for noodles and products, livestock meat, and eggs; < -0.20 mainly for coarse cereals, green leafy vegetables, dried fungi and algae, milk Group and nuts, which explained the 65.23% variation in food and 56.38% variation in response variables. The correlation index of mode 3 factors was > 0.20 mainly for livestock meat; < -0.20 for soybeans, which explained the 5.63% variation in food and 19.35% variation in response variables. As the explanation variation of mode 3 was relatively small, it was excluded.
Table 2
The load of each food component in hHcy-related dietary pattern and its correlation with dietary pattern score

| Food group               | Pattern 1 |            | Pattern 2 |            | Pattern 3 |            |
|--------------------------|-----------|------------|-----------|------------|-----------|------------|
|                          | factor    | correlation| factor    | correlation| factor    | correlation|
|                          | loading   | index      | loading   | index      | loading   | index      |
| Noodles and products     | -0.077    | -0.142c    | 0.231a    | 0.564c     | 0.015     | 0.057      |
| Coarse cereals           | -0.023    | -0.048     | -0.316a   | -0.222c    | -0.040a   | -0.048     |
| Green leafy vegetables   | -0.222a   | -0.160c    | -0.233a   | -0.559c    | 0.040     | 0.014      |
| Dark vegetables          | -0.261a   | -0.113b    | 0.046     | -0.426c    | 0.010     | 0.082      |
| dried fungi and algae    | 0.143     | 0.131c     | -0.232a   | -0.480c    | 0.022     | 0.010      |
| High energy fruits       | 0.130     | 0.600c     | -0.056    | -0.505c    | -0.021    | 0.059      |
| Poultry meat             | 0.311a    | -0.168c    | 0.087     | 0.470c     | 0.038     | 0.029      |
| Livestock meat           | 0.251a    | -0.030     | 0.269a    | 0.461c     | 0.237     | 0.239c     |
| Fish                     | 0.160     | 0.244c     | 0.041     | -0.386c    | -0.080    | 0.188c     |
| Shrimp                   | -0.242    | 0.070      | 0.062     | -0.273c    | 0.022     | 0.005      |
| Eggs                     | 0.178     | -0.048     | 0.291a    | 0.515c     | 0.066     | 0.065      |
| Milk Group               | -0.074    | -0.256c    | -0.247a   | 0.107b     | 0.020     | 0.064      |
| Soybeans                 | -0.212a   | 0.006      | -0.112    | -0.432c    | -0.281a   | -0.614c    |
| Nuts                     | 0.178     | 0.236c     | -0.577a   | -0.436c    | 0.059     | 0.088c     |
| Explain the proportion   |           |            |           |            |           |            |
| of variation             | Pattern 1 | Pattern 2  | Pattern 3 | summation  |           |            |
| Explain each food group  | 4.250     | 9.512      | 0.821     | 14.583     |           |            |
| Explanatory response     | 0.361     | 0.839      | 0.288     | 1.488      |           |            |
| variable                 |           |            |           |            |           |            |

Note: aOnly food groups with absolute factor load > 0.20 are shown. bP < 0.05, cP < 0.01.

Characteristic analysis of dietary pattern quartile

Compared with the lowest quartile array of pattern 1, the subjects in the highest quartile array of pattern 1 had higher energy intake, higher serum Hcy, lower serum FA and B12, and both showed a linear trend. Compared with the lowest quartile array, the subjects in the highest quartile array were older, had higher pre-pregnancy BMI, higher serum Hcy, and lower serum FA and B12, with a linear trend, but there was no difference in energy intake and weight gain during pregnancy (Table 3).
Table 3
Characteristics of subjects under different quartile scores of hHcy-related dietary patterns

|                      | Pattern 1          | Pattern 2          |   | Pattern 1          | Pattern 2          |   |
|----------------------|--------------------|--------------------|---|--------------------|--------------------|---|
|                      | Q1                 | Q4                 | P | values             | Q1                 | Q4 | P |
| Age                  | 29.254 ± 4.203     | 28.869 ± 4.722     | 0.502 | 28.746 ± 4.512 | 30.508 ± 4.679 | 0.0046 |
| Educational background | 0.051             |                    |   |                    | 0.054             |   |
| Senior high school and below | 77(63.64) | 61(50.41) |                  | 54(44.63) | 69(57.02) |   |
| College degree or above | 44(36.36) | 60(49.59) |                  | 67(55.37) | 52(42.98) |   |
| Gravidity            | 0.062             |                    |   |                    | 0.072             |   |
| Primipara            | 57(47.11)         | 72(59.50)          |   |                    | 70(57.85)         | 55(45.45) |   |
| Multipara            | 64(52.89)         | 49(40.50)          |   |                    | 51(42.15)         | 66(54.55) |   |
| Pregnancy BMI(kg/m²) | 22.978 ± 4.088     | 22.078 ± 3.393     | 0.063 | 21.836 ± 3.488 | 23.804 ± 4.355 | <0.001 |
| Weight gain during pregnancy | 8.863 ± 4.782 | 9.502 ± 4.337 |                  | 7.947 ± 4.082 | 8.930 ± 5.365 | 0.1084 |
| Energy intake(kcal/d)| 1482.1 ± 479.3     | 1523.3 ± 468.7     | 0.002 | 1510.9 ± 508.5 | 1530.0 ± 550.2 | 0.7786 |
| Multivitamin supplement intake | | | | | | |
| dietary FA(ug)       | 516.10 ± 637.70    | 477.20 ± 411.10    | 0.574 | 518.90 ± 537.60 | 498.90 ± 557.90 | 0.778 |
| dietary B12 (ug)     | 2.086 ± 3.370      | 2.952 ± 10.550     | 0.394 | 2.153 ± 2.917   | 2.210 ± 2.980   | 0.882 |
| dietary B6 (mg)      | 1.770 ± 2.070      | 2.010 ± 2.636      | 0.433 | 1.830 ± 1.811   | 1.760 ± 1.866   | 0.768 |
| Serological indicator | | | | | | |
| serum FA (nmol/L)    | 183.6 ± 110.900    | 219.5 ± 82.669     | 0.0008 | 238.7 ± 96.952 | 115.7 ± 62.542 | <0.001 |
| serum B12 (pmol/L)   | 17.752 ± 11.976    | 22.443 ± 9.465     | 0.0046 | 23.883 ± 10.075 | 9.266 ± 6.041 | <0.001 |
| serum Hcy (umol/L)   | 7.344 ± 3.141      | 5.403 ± 1.805      | <0.001 | 5.286 ± 1.337  | 9.577 ± 2.833  | <0.001 |

Note: Categorical variables include educational background and pregnancies, expressed as the number of people (constituent ratio). Continuous variables included age, pre-pregnancy BMI, weight gain during pregnancy, energy intake, intake of multivitamin supplements (FA, B12, and B6), and serological indicators (FA, B12, and Hcy), which were expressed as mean ± standard deviation.

Correlation analysis between dietary pattern and GDM

Logistic regression analysis showed that after adjusting for multiple confounding factors, the score of mode 2 was significantly positively correlated with the incidence of GDM (P < 0.01), and the risk of GDM significantly increased relative to the lowest quartile array and the fourth quartile array (OR = 2.963, 95%CI: 0.939–9.356). However, there was no
significant relationship between the score of mode 1 and the incidence of GDM (P > 0.05). The risk of developing GDM in the lowest and highest quartiles of the scores was OR = 0.480 (95% CI: 0.137–1.684) (Table 4).

Table 4
Logistic regression results of the relationship between hHcy-related dietary pattern score and the prevalence of GDM

| Dietary Pattern Score quartiles | Pattern 1 | Pattern 2 | Pattern 1 | Pattern 2 |
|-------------------------------|-----------|-----------|-----------|-----------|
| Q1                            | 1.000     | 1.000     | 1.000     | 1.000     |
| Q2                            | 0.890(0.461–1.718) | 0.809(0.409–1.600) | 1.894(0.916–3.917) | 1.937(0.911–4.117) |
| Q3                            | 0.578(0.259–1.290) | 0.529(0.230–1.213) | 1.983(0.808–4.867) | 1.983(0.808–4.867) |
| Q4                            | 0.518(0.152–1.771) | 0.480(0.137–1.684) | 2.077(0.812–5.315)^a | 2.963(0.939–9.356)^a |

P values

0.350 0.450 0.005 0.004

Note: Model 1: Age, educational background, number of pregnancies, pre-pregnancy BMI, and pregnancy weight gain were adjusted. Model 2: Energy intake and multivitamin supplement intake (folic acid, B12, and B6) were further adjusted. ^aP < 0.01.

Discussion

In this study, serum Hcy, FA and B12 were selected as response variables, and three hHcy-related dietary patterns were extracted using the RRR method. These three dietary patterns could explain the variation in serum Hcy, FA, and B12 to the greatest extent from the perspective of food. Similar proportions of variation in response variables have been explained in other clinical studies using the RRR method [12,13]. The variation explained by mode 3 was significantly smaller than that of modes 1 and 2; thus, it was excluded. It was found that the scores of modes 1 and 2 were positively correlated with the Hcy level, indicating that these two dietary patterns were closely correlated with the Hcy level; this finding is consistent with that of previous studies. Previous studies have found that Hcy levels are significantly correlated with the risk of GDM, which can significantly increase the risk by 20% [5]. Hcy is a sulfur-containing amino acid, an important intermediate in the methionine metabolism process, and any defects that lead to key enzymes or cofactors can result in methionine cycle problems that affect serum Hcy levels and a unit of carbon metabolism-related vitamins, such as vitamin B6, vitamin B12, folic acid, and betaine, which are important coenzymes in the metabolism process. In recent years, an increasing number of studies have suggested that Hcy is closely related to insulin resistance [14], and hHcy should be included in metabolic syndrome [15]. The mechanism is thought to be a result of Hcy being a vascular damaging amino acid that can induce vascular damage and oxidative stress in pancreatic beta cells, leading to disorders of glucose and lipid metabolism [16].

Mode 2 was characterized by a dietary pattern with a higher intake of noodles and products, livestock meat and eggs, and less intake of coarse cereals, green leafy vegetables, dried fungi and algae, milk and nuts. Among the two hHcy-related dietary patterns obtained, only mode 2 showed a positive correlation with the prevalence of GDM. This is consistent with previous studies that state that the dietary pattern with high intake of fruits and vegetables, Coarse cereals, and milk is rich in one-carbon unit metabolic-related vitamins, such as vitamin B6, vitamin B12, folic acid, and betaine, which can reduce the blood Hcy level [7]. However, insufficient intake can increase Hcy levels [17]. There are two components of folic acid intake during pregnancy: from multivitamins and from food (animal liver, poultry, eggs, beans, and leafy greens). In China, continuous supplementation of folic acid in the first 3 months of pregnancy and during pregnancy to prevent fetal neural tube defects is a major public health project [18] to ensure successful birth and good childcare [19]. This study found that only 23.30% of the patients started taking folic acid 3 months before pregnancy, 76.70% of the patients started...
taking folic acid after being pregnant, and 77.13% continued to take folic acid or multivitamins in the second trimester. The results showed that the awareness of taking folic acid supplements during the perinatal period in the Shanghai area is relatively low, and nutritional education for women of childbearing age needs to be strengthened.

Pattern 1 was characterized by a higher intake of poultry and livestock meat and a lower intake of green leafy vegetables, dark vegetables, soybeans, and shrimp. Poultry meat, livestock meat, and other protein foods are rich in methionine, and a high intake of poultry meat or lack of a carbon-unit metabolism-related vitamin will lead to an increase in serum Hcy concentration, which is consistent with previous studies [17]. A prospective clinical study of 681 patients found a significant correlation between meat dietary patterns and the prevalence of GDM [20]. However, this study did not find a significant correlation between dietary pattern score and the prevalence of GDM, this may be because poultry meat offset the risk of GDM caused by elevated serum Hcy levels in other ways. This may be due to the abundance of choline in poultry meat and livestock meat [21], which is another metabolic pathway of Hcy. It can be determined from the one-carbon unit metabolic pathway table that Hcy produces methionine via two methylation pathways: the folate-dependent pathway and the choline/betaine-dependent pathway [22]. The folate-dependent pathway is well known for supplying methyl, while the choline/betaine-dependent pathway has received little attention. Just as a deficiency of folic acid impedes Hcy methylation, individuals who lack choline also have a reduced ability to methylate Hcy, resulting in hHcy [23]. Choline has been suggested as a candidate nutrient intervention for deficient folate intake or metabolic abnormalities [24,25].

The advantage of this study is that two types of hHcy-related dietary patterns were extracted by the RRR method, which explained the variation in hHcy to the greatest extent, rather than the variation in food. Therefore, if dietary guidance of the population is needed, dietary patterns should be extracted using principal component analysis and other methods. In addition, the relationship between hHcy-related dietary patterns and the incidence of GDM was analyzed, which is of great significance in exploring the relationship between dietary patterns and GDM through Hcy levels. The limitation of this study is that the subjects were from an obstetric clinic of only one hospital and the sample size was small. In addition, dietary surveys may have a recall bias. As a cross-sectional study, the causal relationship between dietary patterns and GDM could not be determined. In conclusion, hHcy-related dietary pattern scores are significantly correlated with the risk of GDM, and the influence of Hcy level by adjusting dietary patterns to increase food intake rich in one-carbon unit metabolism-related vitamins may contribute to the intervention and prevention of GDM.

**Abbreviations**
Declarations

Ethics Approval and consent to participate

This study was approved by the Medical Ethics Committee of the Seventh People's Hospital Affiliated to Shanghai University of Chinese Medicine (ethics batch number: 2019-7th-HIRB-014). All the study participants provided informed consent. We confirm that all methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article. The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions
YL completed OGTT and collected clinical information of pregnant women, and was a major contributor in writing the manuscript. LL completed the detection of all serum index. Min-hui Yi collected clinical information of some pregnant women. SW and GL completed OGTT questionnaire. CS checked the content of discussions and data. All authors read and approved the final manuscript. HW summarized and analyzed all data, and was a major contributor in writing the manuscript.

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Tables

Table 1. Comparison of basic characteristics between the GDM and normal groups
### Essential characteristics

|                           | OGTT                                                                 | \( t \)-value | \( P \)-value |
|---------------------------|----------------------------------------------------------------------|----------------|---------------|
|                           | normal group (n=345)                                                 |                |               |
|                           | GDM group (n=143)                                                   |                |               |
| Age                       | 28.513±4.447                                                        | 4.739          | <0.001        |
| Educational background    |                                                                      | -0.576         | 0.565         |
| Senior high school and below | 188 (54.50)                                                          |                |               |
| College degree or above   | 157 (45.50)                                                         |                |               |
| Gravidity                 |                                                                      | -2.475         | 0.013         |
| Primipara                 | 180 (52.17)                                                         |                |               |
| Multipara                 | 165 (47.83)                                                         |                |               |
| Pregnancy BMI(kg/m\(^2\)) | 22.097±3.629                                                        | 4.450          | <0.001        |
| Weight gain during pregnancy(kg) | 8.774±4.144                                                          | 0.045          | 0.964         |
| Energy intake\(\text{kcal/d}\) | 1464.085±411.720                                                   | 3.103          | 0.002         |
| Multivitamin supplement intake |                                                                |                |               |
| dietary FA (ug)           | 538.936±572.832                                                     | 1.998          | 0.028         |
| dietary B12 (ug)          | 2.631±6.828                                                         | 0.204          | 0.838         |
| dietary B6 (mg)           | 1.974±2.288                                                         | 0.862          | 0.389         |
| Serological indicator     |                                                                      |                |               |
| serum FA (nmol/L)         | 21.967±10.523                                                       | 2.877          | 0.004         |
| serum B12 (pmol/L)        | 219.294±110.621                                                     | 2.997          | 0.003         |
| serum Hcy (umol/L)        | 5.770±1.668                                                         | 3.423          | <0.001        |

Note: Categorical variables include educational background and pregnancies, expressed as the number of people (constituent ratio). Continuous variables included age, pre-pregnancy BMI, weight gain during pregnancy, energy intake, intake of multivitamin supplements (FA, B12, and B6), and serological indicators (FA, B12, and Hcy), expressed as mean ± standard deviation, in which energy intake did not include the energy provided by cooking oil intake.

**Table 2.** The load of each food component in hHcy-related dietary pattern and its correlation with dietary pattern score.
| Food group                  | Pattern 1 factor loading | Pattern 1 correlation index | Pattern 2 factor loading | Pattern 2 correlation index | Pattern 3 factor loading | Pattern 3 correlation index |
|----------------------------|--------------------------|-----------------------------|--------------------------|-----------------------------|--------------------------|-----------------------------|
| Noodles and products       | -0.077                   | -0.142<sup>c</sup>         | 0.231<sup>a</sup>       | 0.564<sup>c</sup>          | 0.015                    | 0.057                       |
| Coarse cereals             | -0.023                   | -0.048                      | -0.316<sup>a</sup>      | -0.222<sup>c</sup>         | -0.040<sup>a</sup>       | -0.048                      |
| Green leafy vegetables     | -0.222<sup>a</sup>       | -0.160<sup>c</sup>         | -0.233<sup>a</sup>      | -0.559<sup>c</sup>         | 0.040                    | 0.014                       |
| Dark vegetables            | -0.261<sup>a</sup>       | -0.113<sup>b</sup>         | 0.046                   | -0.426<sup>c</sup>         | 0.010                    | 0.082                       |
| dried fungi and algae      | 0.143                    | 0.131<sup>c</sup>          | -0.232<sup>a</sup>      | -0.480<sup>c</sup>         | 0.022                    | 0.010                       |
| High energy fruits         | 0.130                    | 0.600<sup>c</sup>          | -0.056                  | -0.505<sup>c</sup>         | -0.021                   | 0.059                       |
| Poultry meat               | 0.311<sup>a</sup>        | -0.168<sup>c</sup>         | 0.087                   | 0.470<sup>c</sup>          | 0.038                    | 0.029                       |
| Livestock meat             | 0.251<sup>a</sup>        | -0.030                      | 0.269<sup>a</sup>       | 0.461<sup>c</sup>          | 0.237                    | 0.239<sup>c</sup>           |
| Fish                       | 0.160                    | 0.244<sup>c</sup>          | 0.041                   | -0.386<sup>c</sup>         | -0.080                   | 0.188<sup>c</sup>           |
| Shrimp                     | -0.242                   | 0.070                       | 0.062                   | -0.273<sup>c</sup>         | 0.022                    | 0.005                       |
| Eggs                       | 0.178                    | -0.048                      | 0.291<sup>a</sup>       | 0.515<sup>c</sup>          | 0.066                    | 0.065                       |
| Milk Group                 | -0.074                   | -0.256<sup>c</sup>         | -0.247<sup>a</sup>      | 0.107<sup>b</sup>          | 0.020                    | 0.064                       |
| Soybeans                   | -0.212<sup>a</sup>       | 0.006                       | -0.112                  | -0.432<sup>c</sup>         | -0.281<sup>a</sup>       | -0.614<sup>c</sup>          |
| Nuts                       | 0.178                    | 0.236<sup>c</sup>          | -0.577<sup>a</sup>      | -0.436<sup>c</sup>         | 0.059                    | 0.088<sup>c</sup>           |
| Explain the proportion of variation | Pattern 1 | Pattern 2 | Pattern 3 | summation                   |                          |                             |
| Explain each food group     | 4.250                    | 9.512                       | 0.821                   | 14.583                      |                          |                             |
| Explanatory response variable | 0.361                | 0.839                       | 0.288                   | 1.488                       |                          |                             |

Note: <sup>a</sup>Only food groups with absolute factor load >0.20 are shown. <sup>b</sup> P < 0.05, <sup>c</sup> P < 0.01.

**Table 3.** Characteristics of subjects under different quartile scores of hHcy-related dietary patterns.
|                     | Pattern 1                  | Pattern 2                  |
|---------------------|---------------------------|---------------------------|
|                     | Q1                        | Q4                        | Pvalues | Q1                        | Q4                        | Pvalues |
| Age                 | 29.254±4.203              | 28.869±4.722              | 0.502   | 28.746±4.512              | 30.508±4.679              | 0.0046  |
| Educational background |                          |                           | 0.051   |                           |                           | 0.054   |
| Senior high school and below | 77(63.64)    | 61(50.41)              | 54(44.63) | 69(57.02)           |
| College degree or above    | 44(36.36)    | 60(49.59)              | 67(55.37) | 52(42.98)           |
| Gravidity           |                           |                           | 0.062   |                           |                           | 0.072   |
| Primipara           | 57(47.11)                | 72(59.50)                | 70(57.85) | 55(45.45)           |
| Multipara           | 64(52.89)                | 49(40.50)                | 51(42.15) | 66(54.55)           |
| Pregnancy BMI (kg/m²) | 22.978±4.088          | 22.078±3.393             | 0.063   | 21.836±3.488           | 23.044±4.355             | <0.001  |
| Weight gain during pregnancy | 8.863±4.782    | 9.502±4.337             | 0.276   | 7.947±4.082           | 8.930±5.365             | 0.1084  |
| Energy intake (kcal/d) | 1482.1±479.3           | 1523.3±468.7             | 0.002   | 1510.9±508.5           | 1530.0±550.2             | 0.7786  |
| Multivitamin supplement intake |            |                           |         |                           |                           |         |
| dietary FA (ug)     | 516.10±637.70           | 477.20±411.10            | 0.574   | 518.90±537.60           | 498.90±557.90            | 0.778   |
| dietary B12 (ug)    | 2.086±3.370            | 2.952±10.550            | 0.394   | 2.153±2.917           | 2.210±2.980            | 0.882   |
| dietary B6 (mg)     | 1.770±2.070            | 2.010±2.636             | 0.433   | 1.830±1.811           | 1.760±1.866            | 0.768   |
| Serological indicator |                           |                           |         |                           |                           |         |
| serum FA (nmol/L)   | 183.6±110.900          | 219.5±82.669            | 0.0008  | 238.7±96.952           | 115.7±62.542            | <0.001  |
| serum B12 (pmol/L)  | 17.752±11.976          | 22.443±9.465            | 0.0046  | 23.883±10.017          | 9.266±6.041            | <0.001  |
| serum Hcy (umol/L)  | 7.344±3.141            | 5.403±1.805             | <0.001  | 5.286±1.337           | 9.577±2.833             | <0.001  |

Note: Categorical variables include educational background and pregnancies, expressed as the number of people (constituent ratio). Continuous variables included age, pre-pregnancy BMI, weight gain during pregnancy, energy intake, intake of multivitamin supplements (FA, B12, and B6), and serological indicators (FA, B12, and Hcy), which were expressed as mean ± standard deviation.

**Table 4.** Logistic regression results of the relationship between hHcy-related dietary pattern score and the prevalence of GDM
| Dietary Pattern Score quartiles | Pattern 1 | Pattern 2 |
|-------------------------------|-----------|-----------|
|                               | Model 1   | Model 2   | Model 1 | Model 2 |
| Q1                            | 1.000     | 1.000     | 1.000   | 1.000   |
| Q2                            | 0.890(0.461-1.718) | 0.809(0.409-1.600) | 1.894(0.916-3.917) | 1.937(0.911-4.117) |
| Q3                            | 0.578(0.259-1.290) | 0.529(0.230-1.213) | 1.983(0.808-4.867) | 1.983(0.808-4.867) |
| Q4                            | 0.518(0.152-1.771) | 0.480(0.137-1.684) | 2.077(0.812-5.315) | 2.963(0.939-9.356) |

Note: Model 1: Age, educational background, number of pregnancies, pre-pregnancy BMI, and pregnancy weight gain were adjusted. Model 2: Energy intake and multivitamin supplement intake (folic acid, B12, and B6) were further adjusted. \(^a\)P < 0.01.