Dietary inflammatory index, inflammation biomarkers and preeclampsia risk: A hospital-based case-control study

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This peer-reviewed article has been accepted for publication but not yet copyedited or typeset, and so may be subject to change during the production process. The article is considered published and may be cited using its DOI
10.1017/S0007114522001489
The British Journal of Nutrition is published by Cambridge University Press on behalf of The Nutrition Society

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Abstract

This study evaluated the association between inflammatory diets as measured by the dietary inflammatory index (DII), and inflammation biomarkers, and the development of preeclampsia among the Chinese population. We followed the reporting guidelines of the STROBE statement for observational studies. A total of 466 preeclampsia cases aged over 18 years were recruited between March 2016 and June 2019, and 466 healthy controls were 1:1 ratio matched by age (± 3 years), week of gestation (± 1 week), and gestational diabetes mellitus. The energy-adjusted DII (E-DII) was computed based on dietary intake assessed using a 79-item semiquantitative food frequency questionnaire (FFQ). Inflammatory biomarkers were analyzed by ELISA kits. The mean E-DII scores were -0.65 ± 1.58 for cases and -1.19 ± 1.47 for controls (*P* value <0.001). E-DII scores positively correlated with IFN-γ (r_s = 0.194, *P* value = 0.001) and IL-4 (r_s = 0.135, *P* value = 0.021). After multivariable adjustment, E-DII scores were positively related to preeclampsia risk (*P* trend <0.001). The highest tertile of E-DII was 2.18 times the lowest tertiles (95% CI = 1.52, 3.13). The odds of preeclampsia increased by 30% (95% CI= 18%, 43%, *P* value <0.001) for each E-DII score increase. The preeclampsia risk was positively associated with IL-2 (OR = 1.07, 95% CI = 1.03, 1.11), IL-4 (OR = 1.26, 95% CI = 1.03, 1.54) and TGF-β (OR = 1.17, 95% CI = 1.06, 1.29). Therefore, proinflammatory diets, corresponding to higher IL-2, IL-4 and TGF-β levels, were associated with increased preeclampsia risk.

Keywords: Energy-adjusted dietary inflammatory index; E-DII; Inflammation; Preeclampsia

Introduction

Preeclampsia is one of the gestational complications that leads to maternal and neonatal morbidity and mortality [1]. It has been reported that approximately 2-8% of first pregnancies are diagnosed with preeclampsia [2]. Pregnant woman with preeclampsia generally has a higher risk of adverse outcomes. For instance, respiratory distress syndrome and pneumonia in newborn infants have been reported in a Chinese large-prospective cohort study [3]. In addition, there were about 2.57 times of odds of preterm birth in women with preeclampsia [4].

The etiology of preeclampsia has not yet been fully clarified; however, the hypothesis is that a deficiency of restoring uterine spiral arteries from incomplete placental implantation is the primary causative factor of this disorder [2]. This disruption promotes placental ischemia and contributes to more cellular oxidative stress which is reflected by the release of soluble fms-like tyrosine kinase-1 (sFlt-1) and proinflammatory cytokine such as tumor necrosis factor alpha (TNF-α). Moreover, studies found that the shift toward M1 phenotype leads to strong synthesis of proinflammatory cytokines that would enhance trophoblastic apoptosis [5]. The overexpression of Th1 proinflammatory cytokines such as TNF-α, IFN-γ and IL-2 is associated with preterm delivery and intrauterine growth retardation [6, 7]. The upregulation of other proinflammatory cytokines such as interferon- γ (IFN-γ), interleukin (IL)-2, IL-17, and interferon (IFN-γ), and the downregulation of anti-inflammatory cytokines including IL-4, IL-10, and TGF-β, are eventually detected in preeclampsia [8-10].

More recent studies have focused on some interactions between nutrients and the possible impact of overall dietary habits on chronic systematic inflammation. Higher consumption of dietary fiber, vitamins (e.g., vitamin C, vitamin A, vitamin D), minerals (e.g., calcium, phosphorus, potassium, magnesium), and mono- and polyunsaturated fatty acids are examples of antioxidant nutrients [11], while saturated fatty acids and cholesterol have some inflammation-promoting properties [12]. Unfortunately, since we eat foods as a mixture of numerous nutrients, only focusing on some particular nutrients and diseases may miss much crucial information about their casual relationships. In fact, a systematic review and

meta-analysis found that adequate vegetable and fruit intake could lower the risk of preeclampsia and reduce proteinuria [13].

Based on the inflammatory properties of nutrients, the Dietary Inflammatory Index (DII) was first developed in 2014 by Shivappa et al. [14] to evaluate the inflammatory properties of the diet from maximally anti- to proinflammatory by using a variety of dietary assessment tools such as 24-hour recall, food frequency questionnaires, or 3-day food records [15, 16]. Saturated fatty acids, retinol, processed meat, full-fat dairy, refined grain, sweets, desserts, carbonated beverages and sugar-sweetened beverages are examples of proinflammatory foods with a higher DII score [17, 18]. Nuts, fruits, vegetables, whole grains, fish, and olive oil are characterized as anti-inflammatory foods [17]. Higher DII scores and proinflammatory diets were reported to be associated with 2.12 times higher odds of miscarriage, with a corresponding increase in IL-6 [19].

Recent studies have been published on the association between the dietary inflammatory index and the risk of inflammatory mediated noncommunicable chronic diseases as well as mortality [20]. Based on our knowledge, however, no study has evaluated the potential of diets that are classified by the overall energy-adjusted dietary inflammatory index (E-DII) on the risk of preeclampsia. Therefore, this study aimed to explore the association between inflammatory diets as measured by the energy-adjusted dietary inflammatory index (E-DII) and the development of preeclampsia in a hospital-based population among the Chinese population in Henan province.

Methods

Study population and study design

This study was performed between March 2016 and June 2019 at the First Affiliated Hospital of Zhengzhou University, China. Full details about the study design have been published in previous studies [21-23]. In particular, a total of 466 cases with a ratio of 1:1 matched for case control, were enrolled. The inclusion criteria were women aged over 18 years with at least 28 weeks of gestation with a singleton pregnancy. The presence of preeclampsia among the cases was defined on China's 'Diagnosis and treatment guideline of hypertensive disorders in pregnancy, 2015' guideline [24]. According to this guideline, cases of incident preeclampsia were identified as having systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg after the 20th week of pregnancy and met any of the following criteria: (1) urinary protein ≥ 0.3 g/24 h, urinary protein/creatinine ratio ≥ 0.3, or random urinary protein ≥ (+) (as the examination for quantitative urine protein cannot be carried out in pregnant women); (2) nonalbuminuria but with damage to organs or systems such as the heart, lung, liver, kidney, and other important organs, or abnormal changes in the blood system, digestive system, nervous system, placental-fetal involvement.

The controls recruited corresponding to the cases were women who were preparing for delivery, had never been diagnosed with hypertension or albuminuria, and were matched with the cases for age (± 3 years), week of gestation (± 1 week), and gestational diabetes mellitus (GDM). The following exclusion criteria were applied to both groups: (1) being diagnosed with heart disease, malignancy, hyperthyroidism, immune system diseases, chronic renal insufficiency, and other endocrine system diseases; (2) having epilepsy, depression, and other mental or cognitive dysfunction.

Ethics

The study protocol including other study-related documents was run under the Declaration of Helsinki guidelines and was approved by the Ethics Committee of Scientific Research and Clinical Trials of the First Affiliated Hospital of Zhengzhou University. The informed consent

form for participation in the study was signed by all participants before any epidemiological data and biological specimens were obtained. Data collection followed the reporting guidelines of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement for observational studies.

Dietary assessment

A validate and reproducible 79-item semiquantitative food frequency questionnaire (FFQ) was used to collect information about the participants diet during the past 3 months prior to the study period [25]. The definition of the food groups has been described in detail in a previous study [25]. Each food item asked participants to report how often, on average, they had consumed it for frequency range from never, per day, per week, and per month. A photobook with different food portion sizes was provided for more precisely estimating the quantity of food intake. Nutrients and energy analysis were derived by the China Food Composition Tables 2009, which includes the nutrient portion and energy of each food item [26].

Dietary inflammatory index

To compute the dietary inflammatory index (DII) score, we used the method described in Shivappa et al. [14]. Briefly, the literature published from 1950 to 2010 with 1,943 articles determined the association of 45 food parameters in total for at least one of six inflammatory factors. Scores of -1, +1, and 0 were assigned if the effects were significantly decreased, increased, or nonsignificant changes, respectively, of inflammatory biomarkers including IL-1β, IL-6, TNF, or C-reactive protein. Energy-adjusted dietary inflammatory index (E-DII) scores were calculated using the nutrient density method in which all food parameters were converted to per 1000 kcal of nutrients. In this study, 35 available food parameters from the FFQ were used to calculate the E-DII score and included the following nutrients: energy, carbohydrate, protein, total fat, cholesterol, fatty acids, saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), omega-3 fats, omega-6 fats, soluble fiber, carotene, β-carotene, total carotenoids, thiamin, riboflavin, niacin, vitamin C, vitamin A, vitamin

E, vitamin D, vitamin B6, vitamin B12, folic acid, magnesium, iron, zinc, selenium, total anthocyanidins, total flavonoids, total flavan-3-ol, total flavanones, total flavones and total flavonols. Higher E-DII scores indicate a more proinflammatory diet, whereas lower scores represent a more anti-inflammatory diet.

Laboratory evaluation

A 12-hour overnight fasting blood sample was collected from all participants. The serum was obtained by centrifugation at 3,000 rpm at 4 ℃ for 15 min and stored at -80 ℃ for further analysis [27]. Inflammatory biomarkers were measured in this study using ELISA kits, including TNF-α, IFN-γ, TGF- α, and ILs -2, -4, -10, and -17A. The Th1/Th2 and Th17/Treg ratios were also determined.

Assessment of covariates

Demographic characteristics included age, gestational age (weeks), education level (primary school or less, secondary/high school, college/university or above), and average monthly household income (≤2000, 2000-4000, 4000-6000, >6000 RMB). Lifestyle habits included passive smoking (yes or no), alcohol drinking status and dietary supplements used during the 3 months before pregnancy (yes or no), and physical activity (MET-h/day). Medical conditions, including pre-pregnancy body mass index (kg/m^2) and blood pressure (systolic blood pressure and diastolic blood pressure, mmHg), were collected via face-to-face interview of each case or control.

Statistical analysis

Descriptive statistics were used to summarize the characteristics of the participants. Continuous normally distributed variables are expressed as the mean ± S.D. The Kolmogorov-Smirnoff test was used to test the normality of the data. Skewed distribution continuous variables are expressed as medians and interquartile ranges. Categorical variables are expressed as frequencies or percentages. The general characteristics of the cases and controls were compared using a paired t-test for continuous variables with a normal distribution, whereas the Wilcoxon

signed-ranks test was used for nonnormally distribution. The Pearson chi-square test was applied to compare all categorical data. Tertiles of E-DII scores were calculated based on the distribution of E-DII among controls. For every distribution of the food groups, energy was adjusted prior to comparison across tertiles of the E-DII and the Kruskal-Wallis H test was used. The correlation among nutrients, E-DII, and inflammatory biomarkers was performed by Spearman's correlation coefficient. Binary logistic regression analysis was performed to estimate the association between inflammatory markers and preeclampsia risk. To determine the risk of preeclampsia, the E-DII was analyzed as both a categorical and a continuous variable, and the odds ratios (ORs) and 95% CIs were estimated using conditional logistic regression with different models. The first model was adjusted for age. The second model was fully adjusted for age, gestational age (weeks), pre-pregnancy BMI (kg/m^2), passive smoking (yes or no), alcohol drinking status and dietary supplements used during the 3 months before pregnancy (yes or no), physical activity (MET-h/day) and energy intake (kcal/day).

All tests were two-sided, and a *P* value <0.05 was considered statistically significant. Data were analyzed using SPSS version 26.0 (IBM Corp, Armonk, NY).

Results

The mean age was 30.88 ± 5.00 and 31.00 ± 4.85 in the case and control groups, respectively (Table 1). Cases had significantly higher E-DII scores than controls, of which the means and standard deviations (SDs) were -0.65 ± 1.58 and -1.19 ± 1.47, respectively (*P* value <0.001) (Table 1). Cases with preeclampsia were statistically more likely to have a higher pre-pregnancy BMI, systolic pressure, and diastolic pressure. Furthermore, compared with the controls, cases with preeclampsia were more likely to have primary school or less and were also passive smokers and active alcohol drinkers (Table 1).

With respect to the controls, subjects with preeclampsia had significantly higher IL-4 (2.05 ± 1.51 vs. 1.64 ± 1.09, *P* value = 0.008) and TGF- β (12.43 ± 7.77 vs. 9.13 ± 4.72, *P* value = 0.004) levels (Table 2). Moreover, the Th1/Th2 ratios in preeclampsia were significantly higher than those in healthy pregnancy for both TNF-α/IL-10 ratios (1.99 ± 2.11 vs. 0.68 ± 9.25, *P*

value = 0.039) and IL2/IL-10 ratios (1.95 ± 1.91 vs. 0.59 ± 6.12, *P* value = 0.035) (Table 2). The levels of proinflammatory cytokines, including TNF-α, IFN-γ, IL-2, and IL-17, were not significantly different between preeclampsia cases and controls (Table 2).

While the consumption of total grains significantly increased across tertiles, the consumption of leafy vegetables, starchy vegetables, and fruits as well as fish and seafood were significantly decreased in the preeclampsia cases (*P* value <0.001) (Table 3). Whole grain consumption also decreased across the tertiles of E-DII scores, nevertheless, no significant difference was observed. Similar trends in the consumption of total grains across tertiles were found in the controls (*P* value <0.001) (Table 2). In contrast to preeclampsia cases, with respect to the first tertiles of the E-DII scores, healthy pregnancy in the third tertiles of the E-DII scores had significantly lower whole-grain, poultry, and red meat intake (*P* value <0.001) (Supplementary Table 1).

Regarding the controls, subjects with preeclampsia had significantly greater consumption of tubers, whereas consumption of leafy and starchy vegetables, nuts and seeds, fruits, dairy and products, eggs, fish and seafood, poultry, and red meat were significantly lower (all *P* values <0.05) (Supplementary Table 2). In addition, the lower levels of soluble fiber, protein, cholesterol, Ca, Mg, Fe, Zn, Se, β-carotene, vitamin A, C, D, E, B6, and B12, as well as folate and total flavonoids in preeclampsia were extremely significantly different from those in healthy pregnancies (all *P* values <0.05) (Supplementary Table 2).

The correlations among the E-DII score, inflammatory biomarkers, food groups, and nutrients are shown in Supplementary Tables 3 and 4. E-DII scores showed a significant positive correlation with IFN-γ (r_s = 0.194, *P* value = 0.001) and IL-4 (r_s = 0.135, *P* value = 0.021). The correlation between E-DII scores and food groups revealed that while increasing intake of total grains provided significantly higher E-DII scores (*P* value <0.001), whole grains, leaves, and starchy vegetables, nuts and seeds, fruits, eggs, fish and seafood, poultry, and red meat were significantly inversely correlated with the E-DII scores (*P* value <0.001). In particular, total fat, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, Se, and vitamin E

presented significantly higher E-DII scores (all *P* values <0.05). Th1 cytokine profiles such as TNF-α, IFN-γ, IL-2 and IL-17A were negatively correlated with leafy vegetables, fish and seafood, poultry, soluble fiber, Mg, β-carotene, vitamin A, C, B6, folate, total anthocyanidins and total flavonoids at a significant level (all *P* values <0.05).

Participants whose E-DII belonged to the highest tertile, the most proinflammatory group, were 2.10 times more likely to have preeclampsia than those in the lowest tertiles, the most anti-inflammatory group (age-adjusted OR = 2.10, 95% CI = 1.53, 2.89, *P* trend <0.001) (Table 4). Similarly, when further adjusting was conducted for all possible covariances, there was a 2.18-fold risk of being preeclampsia for those in the highest tertile of E-DII compared to those in the lowest tertiles (full-adjusted OR = 2.18, 95% CI = 1.52, 3.13, *P* trend <0.001) (Table 4). Significant positive associations between the continuous E-DII score and preeclampsia risk in the age-adjusted model and full-adjusted model were simultaneously observed (age-adjusted OR = 1.26, 95% CI = 1.16, 1.38, *P* value <0.001; full-adjusted OR = 1.30, 95% CI = 1.18, 1.43, *P* value <0.001) (Table 4).

The ORs, 95% CIs and *P* values for the preeclampsia risk of inflammatory biomarkers are given in Table 5. After full adjustment for confounding factors, for every one-unit (mg/dL) increase in IL-2, IL-4 and TGF-β, there was a significant corresponding increase in the risk of developing preeclampsia by 7% (95% CI = 3%, 11%, *P* value = 0.001), 26% (95% CI = 3%, 54%, *P* value = 0.024) and 17% (95% CI = 6%, 29%, *P* value = 0.002), respectively. However, TNF-α, IFN-γ, IL-10 and IL-17A were not associated with preeclampsia risk.

Discussion

Our case-control study indicated a positive association between the energy-adjusted dietary inflammatory index (E-DII), as well as inflammation markers (e.g., IL-2, IL-4 and TGF-β), and the risk of preeclampsia. Considering that the E-DII is a sensitive index for inflammatory markers, it can be inferred that lowering the intake of proinflammatory foods with high E-DII scores and increasing the consumption of anti-inflammatory foods with low-EDII scores would be negatively associated with the development of preeclampsia.

Recent studies found some relationship of different food types and intakes amount to pregnancy-related adverse outcomes [20]. Our study is consistent with others that found when compared with the lowest quartile, participants who were more adherent to an unhealthy dietary pattern had a higher risk of preeclampsia, while those in the top quartile of adherence to a healthy dietary pattern had a decreased preeclampsia risk [28]. In this study, an unhealthy dietary pattern was defined as consuming high amounts of processed meat, high GI foods, potatoes, legumes, high-fat dairy, whole-grain, and soft drinks, whereas the healthy dietary pattern included more vegetables, poultry, red meat, eggs, and unsaturated fat [28]. A systematic review and meta-analysis showed that frequently consuming vegetables, fruits, legumes, and whole-grains significantly reduced adverse pregnancy outcomes [29]. Similarly, high plant-based food, and vegetable oil consumption decreased preeclampsia risk, while processed meat, salty snacks, and sweet drinks were positively correlated with the odds of preeclampsia in another study [30]. Nevertheless, a cluster randomized controlled trial conducted in northwestern China concluded that only vegetable-type dietary patterns were inversely related to preeclampsia [31].

All of these publications emphasized the relationship between diet and the development of preeclampsia through a crucial mediator called inflammation. Levels of serum inflammatory biomarkers such as C-reactive protein (CRP), interleukin (IL)-2, IL-4, IL-10, IL-17A, tumor necrosis factor alpha (TNF-α), transforming growth factor beta (TGF-β), interferon (IFN- γ), adiponectin, etc., are able to indicate the level of systematic inflammation and explain the pathogenesis of diseases [32].

The etiology of preeclampsia is hypothesized to involve chronic immune activation or oxidative stress, which is attributed to impaired implantation, leading to an imbalance in the production of proinflammatory and regulatory cytokines. This imbalanced secretion during the gestational period might be the primary causative factor of preeclampsia. In general, regulatory T cell (Treg) proliferation triggered by TGF-β is induced by natural killer cells, resulting in increased maternal tolerance toward the fetus [33]. In addition, M2 phenotype macrophages promote the releases of the Th2 cytokines TGF-β, IL-4, and IL-10 which occurs in successful

pregnancy [33]. In contrast, pregnancies with preeclampsia have limited trophoblast invasion that leads to narrow blood vessels, developing ischemia and hypoxia in the placenta. Moreover, the activation of Th1 cells through the production of TNF-α, IL-6, and IFN-γ also occurs in preeclampsia [33]. Apart from that, the expansion of Th1 and Th17 cells is responsible for triggering inflammation. Among women with a healthy pregnancy, Th1/Th17 activity is suppressed by the proliferation of Tregs; however, this activity seems to be higher in preeclampsia [34].

Previous studies, including a systematic and meta-analysis study, have observed associations between cytokines and the risk of preeclampsia, indicating women are at a higher risk of preeclampsia when their proinflammatory cytokines TNF-α, IFN-γ, IL-2, and IL-17 are elevated and their anti-inflammatory cytokine IL-4, IL-10, TGF-β are depressed [35, 36]. These results might be attributed to the depletion of Th2 cells and Tregs in preeclampsia cases [37]. The decrease in Treg induces apoptosis in trophoblast cells, which limit the invasion of trophoblast cells and remodel the maternal spiral artery [36]. Placental ischemia and hypoxia increase systematic oxidative stress and stimulate pro-inflammatory cytokine secretion as mentioned above.

Moreover, immune cell differentiation and proliferation are influenced by the presence of cytokines, for instance, the presence of IL-6 promoted Th17 cell differentiation while inhibiting Treg cells [38]. At the same time, the IL-17 cytokine produced by Th17 cells could increase the production of IL-6 cytokines [39]. These findings are partly in line with our study, which also showed a significant positive relationship between IL-2 and preeclampsia. However, the association between IL-4 and TGF-β and preeclampsia found in this study was inconsistent with other studies: increasing IL-4 and TGF-β significantly corresponded to an increase in preeclampsia. Since the serum levels of cytokines vary, depending on the condition of the pathology [40], these contradictory reports are probably due to differences in gestational age at sampling, differences in sample size, the severity of the condition, population diversities among

studies, and variations in blood collection and laboratory processing. Moreover, the half-life of the cytokines could also contribute to discrepancies among studies [41].

To provide a further explanation for these findings, dietary inflammatory index (DII) scores were adapted to explain the underlying strategies, since DII scores are based on dietary datasets collected from a dietary survey among the participants in that study, hence they could reflect the activation of oxidative stress and the inflammatory immune response related to specific food parameters [42]. The production of TNF-α, IFN-γ, ILs-2, and -6 is mediated by proinflammatory diets high E-DII foods, corresponding with higher intake of refined grains, dairy and products, red meats, soft drinks, sweets, and desserts, and lower consumption of whole grains, vegetables and fruits [43-45]. Nevertheless, the consumption of red meat across tertiles E-DII among cases in this study was parallel. The effects of red meat and dairy products on inflammation are still controversial. A systematic review and meta-analysis of randomized controlled trials found that high dairy product intake significantly decreased TNF-α and IL-6 levels [11]. Moreover, vegetables and fruits, low E-DII foods, contain antioxidant compounds such as β-carotene, polyphenols, and vitamin C and E. High antioxidants content in these serum and placenta prevent hypoperfusion, thus protecting against preeclampsia development [46]. In addition, flavonoids and β-carotene inhibit the progression of inflammation through the reduction of free oxidative agents and IL-6 and TNF-α production [47, 48].

Some limitations need to be taken into account in the interpretation of our results. First, the present study is a case-control study, and a food frequency questionnaire (FFQ) was used for dietary assessment in this study, so recall bias might exist. Second, since this study is a case-control study, selection bias is inevitable; however, we could reduce differences in sociodemographic information between two groups, ensure the comparability between two groups and reduce the risk of selection bias by recruiting cases and controls from the same referral hospital. Another limitation is that smaller samples with available inflammatory biomarkers were provided in our study; therefore, there is insufficient information about whether inflammation specifically mediates the association between a proinflammatory diet during

pregnancy and preeclampsia. Our results indicate that E-DII scores were associated with greater IFN-γ, which supports the hypothesize that diets induce preeclampsia via systematic inflammation. However, the association between the E-DII and preeclampsia risk remains to be confirmed in prospective analysis.

Conclusion

Individuals with higher E-DII scores and higher IL-2, IL-4 and TGF-β cytokines were associated with increased preeclampsia risk. Additional prospective studies are recommended to confirm our results.

Acknowledgements

We are grateful to the staff at the First Affiliated Hospital of Zhengzhou University in China for technical and material support. This study is funded by National Natural Science Foundation of China (Grant No.81602852).

Y.L. and FZ. contributed to conception and design of the study and manuscript revision. L.Z. wrote the manuscript; C.C. supervised the statistical analyses. S.L. performed E-DII scores and data cleaning. W.F., X.Z., Y.C., W.D., H.C., D.D., and Q.L., made a great contribution to the revised work. All authors have reviewed and approved the manuscript.

The authors have no conflicts of interest to declare.

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Table 1 Characteristics of cases and controls.

Characteristics	Cases (n=466)		Controls (n=466)		*P* value
	Mean/n	S.D./%	Mean/n	S.D./%	
Age (years)	30.88	5.00	31.00	4.85	0.187
Gestational age (weeks)	34.30	2.93	34.36	2.69	0.105
Pre-pregnancy BMI (kg/m^2)	23.80	3.95	22.37	3.35	<0.001
Education level					<0.001
Primary school or less	47	10.1	18	3.9	
Secondary/high school	254	54.5	243	52.3	
College/university or above	165	35.4	204	43.9	
Average monthly household income (RMB/person)					0.092
≤2000	65	14.8	46	10.4	
2000-4000	230	52.3	225	50.9	
4000-6000	82	18.6	87	19.7	
>6000	63	14.3	84	19.0	
Passive smoking					0.193
No	393	84.3	406	87.3	
Yes	73	15.7	59	12.7	
†Drinking status					0.651
No	455	97.6	457	98.1	
Yes	11	2.4	9	1.9	
Dietary vitamin D supplements					0.727
No	460	98.9	460	99.4	
Yes	5	1.1	3	0.6	

Dietary calcium supplements					0.229
No	431	92.7	439	94.6	
Yes	34	7.3	25	5.4	
Dietary calcium plus vitamin D supplements					0.511
No	207	44.4	217	46.6	
Yes	259	55.6	249	53.4	
Dietary folic acid supplements					0.509
No	95	20.4	87	18.7	
Yes	371	79.6	379	81.3	
Dietary iron supplements					0.009
No	423	90.8	397	85.2	
Yes	43	9.2	69	14.8	
Dietary multivitamin supplements					0.702
No	405	86.9	401	86.1	
Yes	61	13.1	65	13.9	
Blood pressure (mmHg)					
Systolic	153.39	16.70	113.56	10.68	<0.001
Diastolic	99.76	12.29	73.13	9.01	<0.001
‡Physical activity (MET-h/day)	26.91	3.91	26.59	4.48	0.258
Total energy intake (kcal/d)	1843.75	503.63	1962.38	527.72	<0.001
E-DII score	-0.65	1.58	-1.19	1.47	<0.001

Abbreviation: S.D., standard deviation; BMI, body mass index.

Test for differences of case and control using a paired *t*-test for normally distributed continuous variables; Wilcoxon signed-ranks test for skewed distributed continuous variables; Chi-square test for categorical variables; *P* value<0.05 indicates significant difference.

Values are presented as mean (S.D.) for continuous variables; number (%) for categorical variables.

[†]Drinking status refers to alcohol consumption during the 3 months before pregnancy.

[‡]Physical activities included daily occupational, leisure-time and household-chores, evaluated by metabolic equivalent (MET) hours per day.

Table 2 Comparison of inflammatory biomarkers between cases and controls

	Cases					Controls					*P* value
	N	Mean	S.D.	Median	IQR	N	Mean	S.D.	Median	IQR	
Th1											
TNF-α (mg/dL)	139	9.40	10.95	7.16	4.70	146	7.78	6.89	5.79	3.09	0.115
IFN-γ (mg/dL)	148	5.25	4.18	4.69	3.49	155	5.06	4.96	4.04	3.82	0.298
IL-2 (mg/dL)	147	9.04	7.44	6.24	8.62	154	6.24	6.33	4.36	4.30	0.074
Th2											
IL-4 (mg/dL)	150	2.05	1.51	1.91	1.11	149	1.64	1.09	1.73	1.14	0.008
IL-10 (mg/dL)	149	6.69	5.90	1.91	4.21	157	7.74	10.22	4.97	5.87	0.927
Th1/Th2											
TNF-α/IL-10	136	1.99	2.11	1.64	1.34	145	0.68	9.25	1.11	1.57	0.039
IL2/IL-10	145	1.95	1.91	1.35	1.80	153	0.59	6.12	0.76	1.39	0.035
Th17											

IL-17A (mg/dL)	151	4.35	3.76	3.53	3.26	146	3.81	4.03	2.93	0.39	0.604
T reg											
TGF-β (mg/dL)	68	12.43	7.77	10.52	3.50	60	9.13	4.72	8.89	4.03	0.004
Th17/T reg											
IL-17A/TGF-β	67	0.44	0.34	0.38	0.37	58	0.42	0.35	0.36	0.36	0.790

Abbreviation: SD, standard deviation; IQR, interquartile range; Th, helper T cells; Treg, regulatory T cells; TNF-α, tumor necrosis factor alpha; IFN-γ, interferon gamma; ILs, interleukins; TGF- β, transforming growth factor beta.

Test for differences of case and control using Wilcoxon signed-ranks test; *P* value<0.05 indicates significant difference.

Table 3 Distribution of food groups across tertiles of energy-adjusted dietary inflammatory index (E-DII) for preeclampsia cases (n=466).

Food groups (g per 1000 kcal)	Tertiles of E-DII						*P* trend
	I (-5.40 to -1.83)		II (-1.83 to -0.63)		III (-0.63 to 3.69)		
	Median	IQR	Median	IQR	Median	IQR	
Total grains	153.29	67.40	183.83	75.07	199.37	80.86	<0.001
Whole grains	7.34	20.26	7.02	15.17	4.99	12.23	0.085
Tuber	31.60	10.51	32.03	9.62	33.76	10.47	0.064
Leaf Vegetables	227.76	124.26	150.21	71.73	101.67	61.83	<0.001
Starchy vegetables	142.70	141.28	130.95	101.01	97.44	75.93	<0.001
Nuts and seeds	10.35	21.45	9.95	18.20	5.42	18.86	0.084
Fruits	355.82	345.86	305.33	179.01	229.97	201.65	<0.001
Dairy and products	140.74	201.61	130.29	208.19	155.16	228.98	0.339
Egg	52.47	50.76	51.65	52.02	51.64	53.92	0.578
Fish and seafood	7.18	15.86	4.90	11.10	3.75	8.54	0.001
Poultry	4.54	10.85	4.32	9.16	4.22	8.26	0.736
Red meat	28.73	44.51	26.40	35.36	27.8	42.79	0.962

Abbreviation: IQR, interquartile range.

Comparison of food groups across tertiles of energy adjusted-dietary inflammatory index (E-DII) by using Kruskal-Wallis *H* test; *P* value<0.05 indicates significant difference.

All foods groups were calculated by edible portions; total grains, whole grains, nuts and seeds were calculated by dry uncooked weights.

Dairy and products included fresh whole and skim cow's milk, yogurt, butter, cheese and ice cream.

Table 4 Odds ratios and 95% confidence intervals for association between the energy-adjusted dietary inflammatory index (E-DII) and the risk of preeclampsia.

	Tertiles of E-DII (OR, 95%CI)					
	I (-5.40 to -1.83)	II (-1.83 to -0.63)	III (-0.63 to 3.69)	*P* trend	E-DII continuous (OR, 95%CI)	*P* value
N (case/control)	109/155	128/156	229/155			
Model 1	1	1.17 (0.83, 1.64)	2.10 (1.53, 2.89)	<0.001	1.26 (1.16, 1.38)	<0.001
Model 2	1	1.15 (0.78, 1.70)	2.18 (1.52, 3.13)	<0.001	1.30 (1.18, 1.43)	<0.001

Abbreviation: OR, odds ratio; CI, confidence intervals.

Crude and adjusted OR and 95%CI were obtained from conditional logistic regression model by entering method; *P* value or *P* trend<0.05 indicates significant difference.

Model 1 = Adjusted for age

Model 2 = Fully adjusted for age, gestational age (weeks), pre-pregnancy BMI (kg/m^2), education level (primary school or less, secondary/ high school, college/ university or above), passive smoking (yes or no), drinking status and dietary supplements used (e.g., vitamin D, calcium, calcium plus vitamin D, iron, folic acid, and multivitamin supplements) during the 3 months before pregnancy (yes or no), physical activity (MET-h/day), and energy intake (kcal/day).

Table 5 Binary logistic regression analysis according to the association between the inflammatory biomarkers and preeclampsia (n=466).

Outcomes	*β*	SE	OR (95%CI)	*P* value
TNF-α (mg/dL)	0.026	0.017	1.03 (0.99, 1.06)	0.116
IFN-γ (mg/dL)	0.003	0.027	1.00 (0.95, 1.06)	0.905
IL-2 (mg/dL)	0.064	0.020	1.07 (1.03, 1.11)	0.001
IL-4 (mg/dL)	0.233	0.100	1.26 (1.03, 1.54)	0.024
IL-10 (mg/dL)	-0.022	0.016	0.98 (0.95, 1.01)	0.163
IL-17A (mg/dL)	0.031	0.032	1.03 (0.97, 1.10)	0.335
TGF-β (mg/dL)	0.154	0.050	1.17 (1.06, 1.29)	0.002

Abbreviation: *β*, beta estimate; SE, standard error; TNF-α, tumor necrosis factor alpha; IFN-γ, interferon gamma; ILs, interleukins; TGF- β, transforming growth factor beta; *P* value <0.05 indicates significant difference.

All values adjusted for age, gestational age (weeks), pre-pregnancy BMI (kg/m^2), education level (primary school or less, secondary/ high school, college/ university or above), passive smoking (yes or no), drinking status and dietary supplements used (e.g., vitamin D, calcium, calcium plus vitamin D, iron, folic acid, and multivitamin supplements) during the 3 months before pregnancy (yes or no), physical activity (MET-h/day), and energy intake (kcal/day).

The *β* estimate represents the change of each outcome per each 1-unit increase in inflammatory biomarkers.