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The relationship between folic acid deficiency and preeclampsia-like phenotypes in rats

Jing Wei[1†], Feilong Lu[2†], Yingya Lou[3], Yanhua Liu[4] and Hongbo Zhai[1*]

Abstract

Background Pre-eclampsia is a significant contributor to maternal and neonatal morbidity and mortality. However, its etiology remains elusive. More and more studies have highlighted the potential involvement of folic acid metabolism in the development of pre-eclampsia. Folic acid is known to be important for DNA synthesis and methylation processes, which are crucial during pregnancy. Disruptions in these pathways may contribute to the pathogenesis of pre-eclampsia. Clinical studies investigating associations between folic acid supplementation and pre-eclampsia produced inconsistent results. The research aims to explore the potential link between folic acid deficiency and the development of pre-eclampsia-like symptoms in rat models, shedding light on the possible role of one-carbon metabolic pathways in the etiology of pre-eclampsia.

Methods Establishing a rat model with severe and moderate folate deficiency by providing female rats with a folate-deficient diet from birth or weaning, respectively. The effects on folate and homocysteine levels during pregnancy were then studied.

Results Both groups exposed to folate deficiency exhibited decreased levels of 5-methyltetrahydrofolic acid in both plasma and red blood cells, along with increased levels of homocysteine in plasma, compared to the control group. Consistent high blood pressure and urinary protein excretion were not significantly different among the three groups. However, fetuses from the folate-deficient group exhibited noticeably lower body weight compared to those from the folate-replete group.

Conclusions Folate deficiency alone may not be sufficient to cause pre-eclampsia in rats, but it does increase the risk of offspring being small for their gestational age at birth.

Keywords Folate, Folic acid, Pre-eclampsia, One-carbon metabolic, Homocysteine

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Introduction

Pre-eclampsia (PE) is defined as the onset of hypertension (systolic blood pressure sustained at ≥ 140 mmHg or diastolic blood pressure sustained at ≥ 90 mmHg, or both) accompanied by proteinuria or end-organ dysfunction, occurring after 20 weeks' gestation. It affects approximately 2–8% of pregnancy women and is one of the.

leading causes of maternal and perinatal morbidity and mortality [1–3].

PE is a progressive multi-system pregnancy disorder characterized by variable degrees of placental malperfusion, resulting in the release of soluble factors into circulation. Numerous candidate factors, including soluble fms-like tyrosine kinase-1 (sFlt1), are excessively secreted by the PE placenta and may contribute to endothelial dysfunction. This event is combined with suppression of the release of pro-angiogenic placental growth factor (PLGF). Placental malperfusion can also result in fetal growth restriction and stillbirth. Currently, there are no effective interventions available to halt disease progression, leaving prompt delivery of the fetus and placenta as the sole option [4].

The etiology of PE remains elusive, in order to study its underlying mechanisms, multiple animal models of pre-eclampsia had been designed, including reduced uterine perfusion pressure (RUPP) [5]; angiogenic and growth factor mutants [6–8]; the N-nitro-L-arginine methyl ester (L-NAME) mutant [9]; and the arginine vasopressin (AVP) infusion model [10]. These models are characterized by placental ischemia, oxidative stress, angiogenic and growth factor changes, inflammation, and interactions between these mechanisms.

Folic acid, a B-group vitamin essential for nucleotide metabolism and crucial for DNA synthesis and methylation, plays a vital role in maintaining normal pregnancy due to its active functions in antioxidant protection, angiogenesis, endothelial-dependent vascular relaxation, and homocysteine remethylation [11]. Clinical studies investigating associations between folic acid supplementation and PE produced inconsistent results [12]. One study found that women who used ≥ 800 μg folic acid supplements had a higher risk of developing gestational hypertension [13]. While, another meta-analysis reported that multivitamin supplementation containing folic acid during pregnancy did not show significant preventive effects on PE [14]. The objective of this study was to evaluate the impact of different degrees of folic acid deficiency on hypertension or PE in pregnant rats.

Materials and methods

Animals diets and collected data

The research reported here adhered to the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines. All rat experiments were approved by the institutional Animal Care and Use Committee of Hangzhou Normal University (HSD20220704). The Six Sprague Dawley (SD) female rats, weighing between 250 g and 280 g, were housed in a specific pathogen-free environment maintained at a temperature range of 18–24℃. They were exposed to a 12-hour light and 12-hour dark cycle while having unrestricted access to food and water. The three SD male rats weighing between 300 g and 320 g were selected and given four days to acclimate before starting feeding and cohabitation. Subsequently, 18 female neonatal rats, all available females, were divided randomly into three groups of 6 rats each: control group (standard diet), moderate deficiency group (standard diet until postnatal day 21, then folate-deficient diet), and severe deficiency group (folate-deficient diet from birth). The allocation process involved assigning numbers to rats, matching those numbers with lots, shuffling the lots in a box, drawing them in sequence, and then randomly selecting groups based on this order. The rat diet was obtained from Jiangsu Xietong Pharmaceutical Bio-engineering Co., Ltd. The only difference between the standard diet and the folate-deficient diet was the inclusion of 2 mg of folic acid per kilogram in the former. The 18 female neonatal rats were allowed to grow until they reached 12 weeks of age, at which point mating occurred. Blood samples were obtained from rats at 3 weeks old, 7 weeks old, and at 0, 10.5, and 19.5 days post coitum (dpc) using orbital blood collection technique. The blood pressure measurements were taken in rats at 10 weeks old, 11 weeks old, and at 7.5, 10.5, and 19.5 dpc. The fetuses were collected at 19.5 dpc after receiving pentobarbital sodium intraperitoneally at a dose of 60 mg/kg. Then the fetal body weight, and crown-to-rump length was meticulously documented. Urine samples were collected from rats at 0 dpc, 10.5 dpc, and 19.5 dpc after the rats spent four hours acclimating to metabolic cages before each collection day. The rats were kept in the same metabolic cages with a collection tube attached to a bottle for a whole day after which urine was collected, while they were given food and water throughout this period (Fig. 1).

Measurements of 5-MTHF and homocysteine (hcy) in the blood by LC-MS/MS

Within 2 h of blood drawing, blood was spun at 4℃ and 2000 g for 10 min, and plasma were prepared. The remaining RBC was added with 2 times volume of saline. The tubes were centrifuged again and the supernatant was discarded. The washed RBC were re-suspended with 2 volumes of saline and 100 ul RBC suspension was withdrawn for the test. The plasma concentration of 5-MTHF and Hcy, and RBC concentrations of 5-MTHF were determined by Liquid Chromatography-Tandem Mass

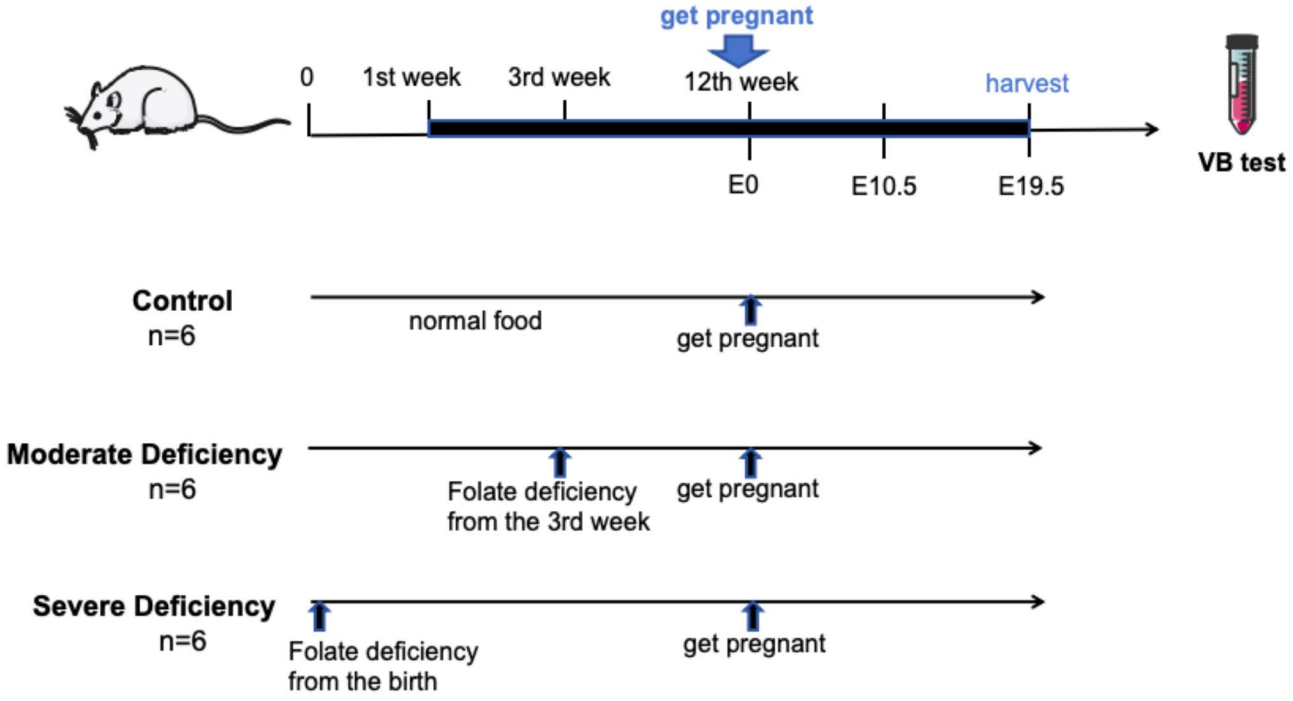

Fig. 1 Experimental schematic

Spectrometry (LC-MS/MS) using Complete Folate-function Test Kit from Vito diagnostics (Hangzhou, China). LC separation was accomplished by a reverse phase column. All data were acquired using the multiple-reaction monitoring mode in the positive ion modes on the AB SCIEX Triple Quad 4500MD mass spectrometer and processed by the Analyst 1.6.3 software. This method was highly sensitive and specific with the lower limit of quantitation of 5-MTHF 4.08 nmol /L, Hcy 1.85 μmol/L in plasma, and 5-MTHF 17.0 nmol/L in RBC.

Measurement of PLGF and sFlt-1 in the plasma

Enzyme-linked immunosorbent assays (ELISAs) for rats sFlt-1 and PLGF were conducted with the use of commercial kits (Jiangsu Longwei Biological Technology) according to the manufacture's instruction. The fluorescence immunoanalyzer automatically analyzes the results and calculates the concentration of target analytes (PLGF, sFlt-1) in each sample; manual calculation is performed to determine sFlt-1/PLGF ratio.

Statistical analysis

Quantitative data are presented as means ± SEM, and statistical significance are reported in the figures and in the figure legends. All experiments were independently repeated at least three times. ANOVA with Tukey's post-test (One-way ANOVA for comparisons between groups, Two-way ANOVA for comparisons of magnitude of changes between different groups) was used to compare values among different experimental groups using the GraphPad program. For experiments with only two groups, Student's test was used as specified in the figure legends. $p < 0.05$ was considered statistically significant (*), $p < 0.01$ as highly significant (**), $p < 0.001$ as extremely significant (***), and ns as not significant.

Results

Blood pressure

No statistically significant difference in systolic blood pressure was observed between the control group and the severe deficiency group at 10 weeks, 11 weeks, 7.5 dpc, 10.5 dpc, or 19.5 dpc (Fig. 2a).

Folate deficiency groups had higher diastolic blood pressure at 11 weeks of age compared to the control group. Furthermore, at 7.5 dpc, the moderate folate deficiency group exhibited higher diastolic blood pressure compared to the control group (Fig. 2b).

At 11 weeks of age, there was a notable increase in mean arterial pressure in the deficiency groups compared to the control group. However, there were no statistically significant differences in mean arterial pressure observed among the three groups at other time points (Fig. 2c).

Homocysteine

The groups with folic acid deficiency and the control group showed notable differences in homocysteine levels at every stage during development and pregnancy (Fig. 3).

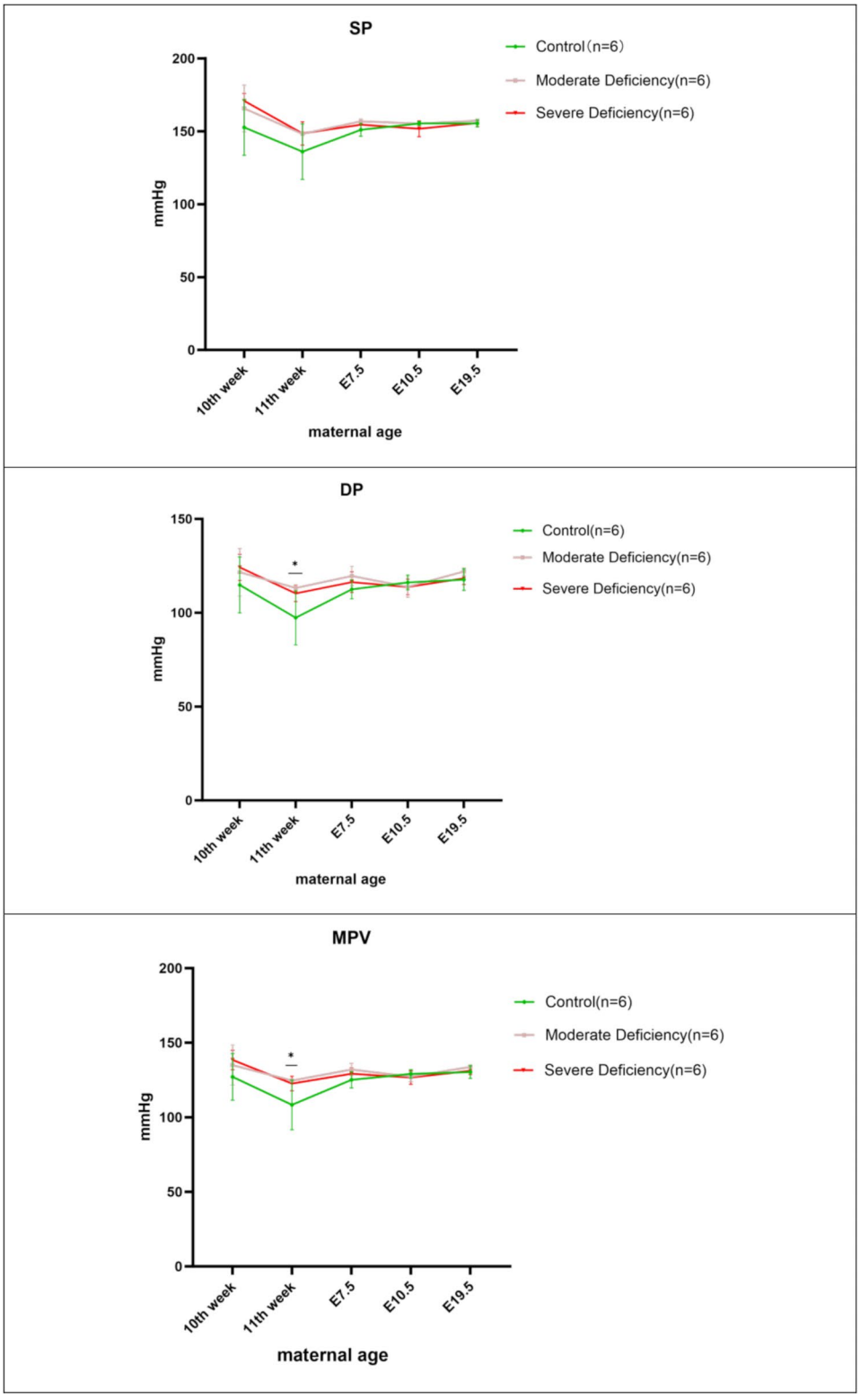

Fig. 2 Blood pressure in five stages among the three groups. (**a**) Systolic blood pressure; (**b**) Diastolic blood pressure; (**c**) Mean arterial pressure

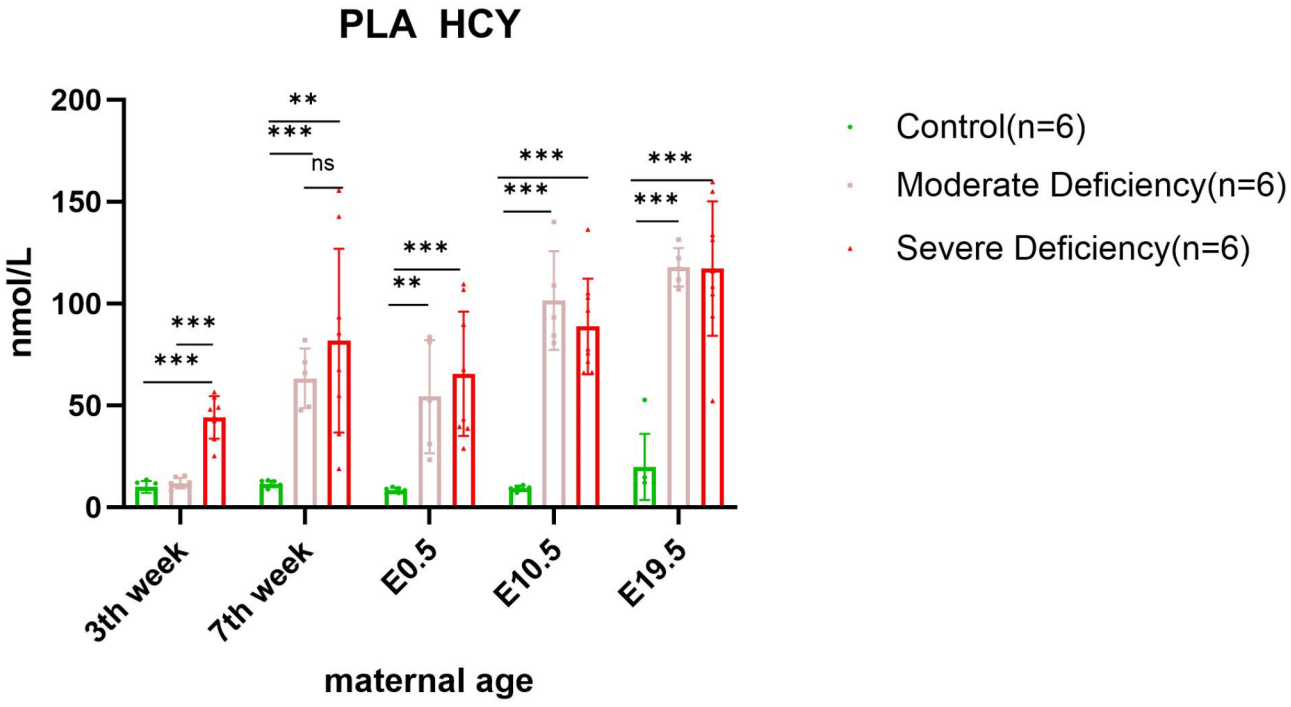

Fig. 3 Homocysteine levels in five stages among three groups

5-methyltetrahydrofolate

The levels of 5-methyltetrahydrofolate in both plasma and red blood cells were significantly lower in the two groups with folic acid deficiency compared to the control group (Fig. 4).

24-hour urinary protein quantity

Increased urinary protein content was only seen in the folate deficient group on day 0 of pregnancy, with no notable differences among the control and folate deficient groups after pregnancy (Fig. 5).

PLGF, sFlt-1 and sFlt-1/PLGF

At 19.5 dpc, there were no statistically significant differences in the levels of PLGF, sFlt-1, and sFlt-1/PLGF between the group with folic acid deficiency and the control group (Fig. 6).

Birth weight and length of mice

The mice at 19.5 dpc from pregnant rats with severe folic acid deficiency showed statistically significant reductions in both body length and weight (Fig. 7).

Discussion

Our attempt to create a rat model of PE using a folate-deficient diet did not succeed. The outcome consistent with Falco et al.'s research [15], indicating that folic acid deficiency by themselves do not lead to the development of PE in animal models.

Folate and PE

In PE, there is impaired trophoblast invasion [16]. Folic acid appears to influence trophoblast invasion, crucial for placental development [17]. Low folic acid levels are linked to PE development, while supplementation may offer protection through epigenetic modifications in the placenta [18].

Several studies have reported that folate supplementation, including folic acid-containing multivitamins, can have protective effects against PE [19–21]. The studies by Shi et al. [19] suggested that supplementation with multivitamins containing folic acid in the second trimester is linked to a decreased risk of PE. Additionally, Zheng et al. [21] found that high-dose folic acid (4 mg/day) from three months before pregnancy throughout gestation can effectively lower the recurrence of PE.

However, there were conflicting findings in the literature. One multicenter clinical trial found that daily intake of 4 mg folic acid between weeks eight and sixteen of pregnancy did not prevent PE in high-risk women [22]. The study conducted by Li et al. [23] found that consuming 400ug of folic acid per day during early pregnancy did not prevent the occurrence of gestational hypertension or PE in a sample of 193,554 women from two southern provinces of China.

Furthermore, it's important to note that high-dose folate supplementation may have adverse effects such as increasing the risk of PE. Studies conducted by Yan et al. [24–25], indicated that excessive folic acid intake in pregnant mice can lead to methylenetetrahydrofolate

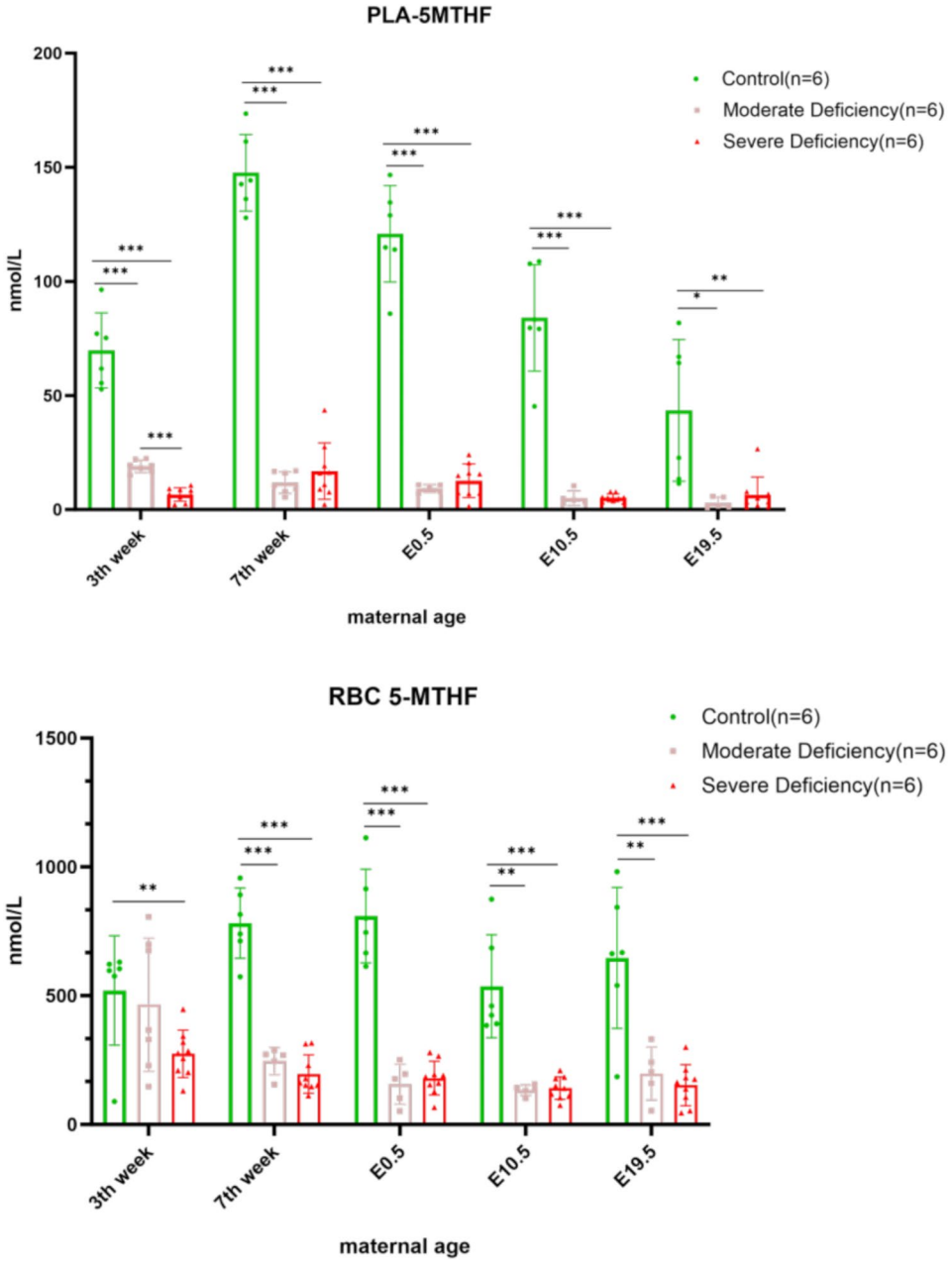

Fig. 4 5-methyltetrahydrofolate levels in both plasma and red blood cells in five stages among the three groups

reductase (MTHFR) deficiency and disrupted choline/methyl metabolism, potentially causing neurobehavioral issues in newborns.

The relationship between homocysteine (Hcy), PE and offspring birthweight

High levels of Hcy in pregnant women have been associated with adverse pregnancy outcomes, including PE, intrauterine growth restriction and lower birth-weights in offspring [26–28]. Yajnik et al. [28]found that Indian mothers with higher Hcy levels had lower birthweight offspring by 56 g/SD increase in Hcy during pregnancy. Comparing lowest versus highest quartile showed potential for more significant effects (differences ranged from 110 to 150 g). Genetic proxies indicated a stronger correlation, with an estimated reduction of approximately 250 g per standard deviation of Hcy concerning birthweight. The study by Generation R found that higher levels of Hcy (in the highest quintile) were associated with lower placental weight (a 30 g difference; $P<0.001$) and reduced birthweight (a 110 g difference; $P<0.001$) compared to individuals in the lowest quintile of Hcy levels [29]. A systematic review and meta-analysis revealed that when maternal Hcy concentrations reached the 90th

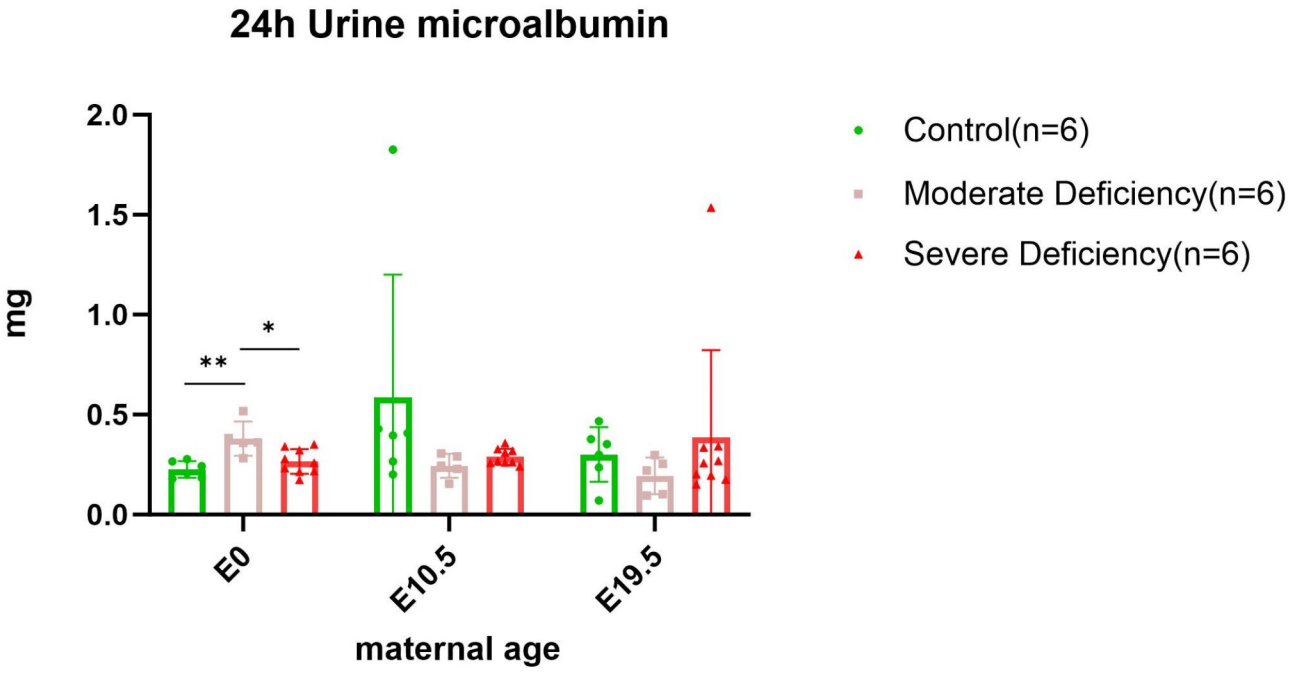

Fig. 5 24-hour urinary protein quantity in three stages among the three groups

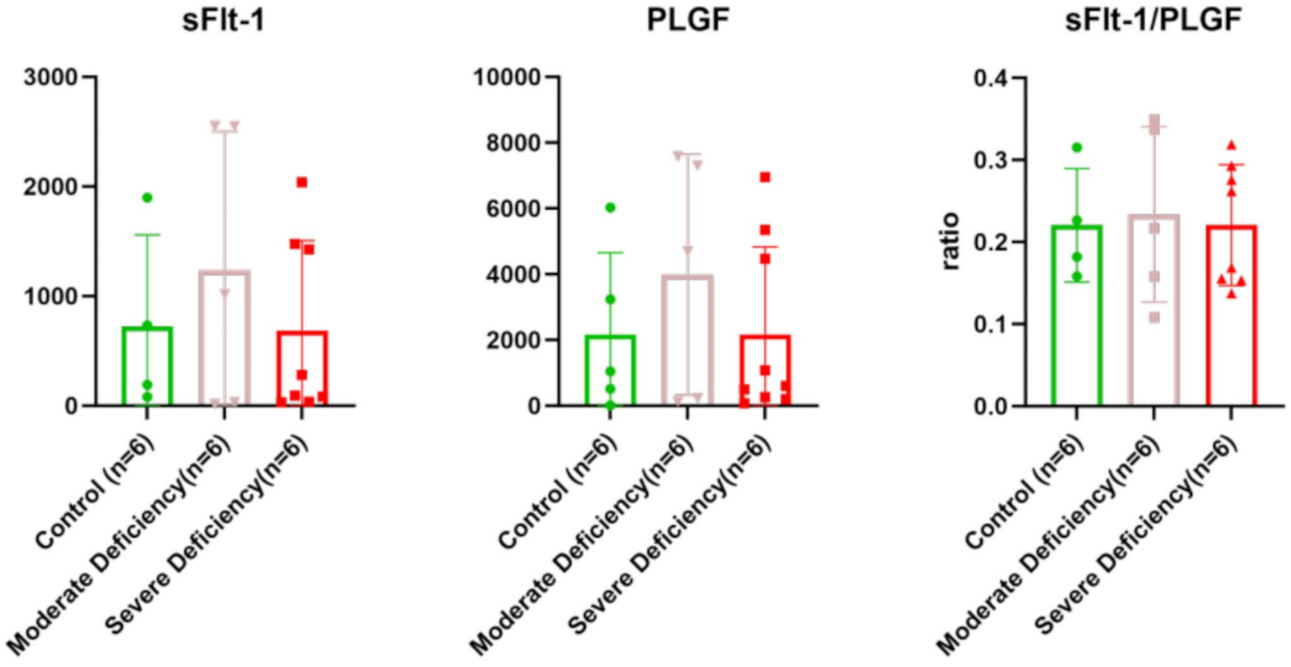

Fig. 6 the levels of PLGF、sFlt-1 and sFlt-1/PLGF in three groups

percentile, there was a 25% increased risk of delivering small-for-gestational-age (SGA) offspring [26].

Other studies have yielded conflicting results. In a prospective study mentioned [30], there was no significant correlation between Hcy levels in early pregnancy and the risk of small size for gestational age at birth. However, a positive correlation was found between Hcy concentration and PE (RR 2.7, 95% CI 1.4–5.0). In a systematic review [31], pregnant women with PE exhibited higher serum Hcy concentrations compared to those with uncomplicated pregnancies. However, no dose-response relationship was observed between Hcy concentration and the severity of PE. Another prospective study [32] reported no significant relationship between Hcy levels and any of the maternal or neonatal outcomes (such as PE, or neonatal birth weight).

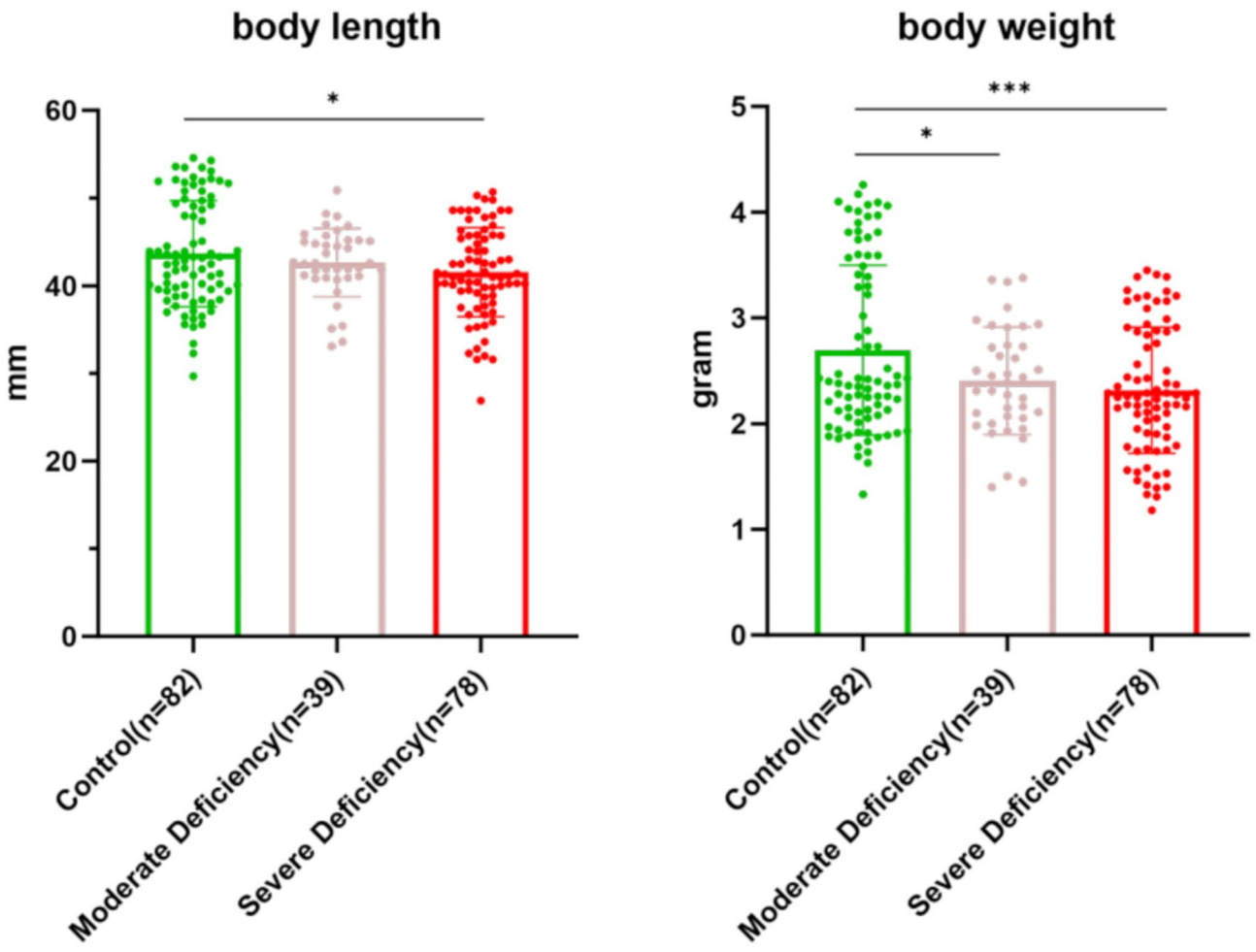

Fig. 7 Length and weight of the 19.5 dpc mice among three groups

Our study found that both groups with folate deficiency showed higher serum Hcy levels compared to the control group, along with decreased birthweight in offspring. Significantly, the group with severe folate deficiency exhibited the highest concentrations of serum homocysteine and the lowest birthweight of offspring among all groups..

The possible mechanisms of these associations include the role of 1-C groups in nucleic acid synthesis, methylation of DNA affecting gene expression regulation, provision of methionine for protein synthesis via 1-C metabolism, and the impact of dietary methyl donors on fetal programming shown in animal studies [33]. Hyperhomocysteinemia may directly damage endothelium by reducing nitric oxide levels, activating pro-inflammatory and oxidative stress pathways, potentially leading to impaired placental perfusion and function [34].

The relationship between PLGF, sFlt-1 and sFlt-1/PLGF ratio

sFlt-1, a circulating antiangiogenic protein that sequesters the proangiogenic proteins PLGF and vascular endothelial growth factor (VEGF), exhibits elevated levels in the circulation of women with PE prior to the onset of clinical symptoms. The sFlt-1 level increased beginning approximately five weeks before the onset of PE. The levels of PLGF were significantly lower in the women who subsequently developed PE compared to the control group, starting from 13 to 16 weeks of gestation. The most pronounced difference was observed during the weeks preceding the onset of PE, coinciding with an increase in sFlt-1 levels [35]. The automated measurement of the sFlt-1/PLGF ratio has a sensitivity of 89% and a specificity of 97% for detecting early-onset PE when using a single, gestation-wide cutoff of 85 [36].

Our study found no statistically significant differences in PLGF, sFlt-1, and sFlt-1/PLGF levels at 19.5dpc between the folic acid deficiency group and the control group. Not conducting follow-up gene expression studies for *sflt-1* and *plgf* is a limitation as it hinders a comprehensive understanding of their role within the study.

Conclusions

This study represents an initial exploration into the potential link between folic acid deficiency and PE-like symptoms in a rat model. In this study, we found that folate deficiency appears to be associated with elevated blood pressure levels compared to folate sufficiency in certain time periods. However, this difference was not consistently observed across all time points, indicating that the elevated blood pressure may have been temporary. It may imply that folate insufficiency may not directly contribute to PE, the direct link between folate insufficiency and PE is not well-established. In this study, we also deeply understand monitoring and managing Hcy levels can be crucial in promoting healthy birthweights in offspring.

In the study, only 18 female mice were selected for further investigation, which is not a large sample size. This could also be considered as a limitation of the study. Further research with large sample size, along with investigating other factors such as inflammatory factors would help determine if folate deficiency truly contributes to elevated blood pressure levels and its implications for PE.

Abbreviations

PE	Pre-eclampsia
Hcy	Homocysteine
5-MTHF	5-methyltetrahydrofolate
MTHFR	Methylenetetrahydrofolate reductase
sFlt1	Fms-like tyrosine kinase-1
PLGT	Placental growth factor
RUPP	Reduceduterine perfusion pressure
L-NAME	Nitro-L-arginine methyl ester
AVP	Arginine vasopressin
SGA	Small-for-gestational-age

Supplementary information

The online version contains supplementary material available at https://doi.org/10.1186/s12884-025-07343-3.

Supplementary Material 1

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Author contributions

JW: conceived and designed the research, wrote the manuscript. FLL: performed the experiments, interpreted results of experiments. YHL: performed the experiments. YYL: collected the data and prepared the figures. HBZ: conceived and designed the research, revised the manuscript. All authors reviewed the manuscript.

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Data availability

The research data used in this paper is publicly available, and we have followed the data usage protocol and relevant regulations for data processing and analysis. In this study, we used standard statistical methods and analysis procedures to preprocess and clean the data. The data that support the findings of this study are available from the corresponding author on reasonable request.

Declarations

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

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