British Journal of Nutrition (2023), 130, 1329–1337 doi:10.1017/S0007114523000314
© The Author(s), 2023. Published by Cambridge University Press on behalf of The Nutrition Society

Serum folate mediates the associations of *MTHFR* rs1801133 polymorphism with blood glucose levels and gestational diabetes mellitus in Chinese Han pregnant women

Yunguo Wang[1]†, Yiyun Wang[2,3]†, Yao Sun[2,3], Naijian Zhang[3], Xiaoshan Liang[3,4], Suhui Luo[3,4], Lirong Dai[5], Chao Sun[5], Yungui Yang[6], Shuying Li[7], Xumei Zhang[3,4] and Qiang Zhang[2,3]*

[1]*Department of Orthopedics Surgery, Second Hospital of Tianjin Medical University, Tianjin Medical University, Tianjin 300211, People's Republic of China*
[2]*Department of Occupational and Environmental Health, School of Public Health, Tianjin Medical University, Tianjin 300070, People's Republic of China*
[3]*Tianjin Key Laboratory of Environment, Nutrition and Public Health, School of Public Health, Tianjin Medical University, Tianjin 300070, People's Republic of China*
[4]*Department of Nutrition and Food Science, School of Public Health, Tianjin Medical University, Tianjin 300070, People's Republic of China*
[5]*Community Health Service Center of Yangliuqing Town, Tianjin 300380, People's Republic of China*
[6]*Community Health Service Center of Zhangjiawo Town, Tianjin 300393, People's Republic of China*
[7]*Department of Endocrinology, Tianjin Xiqing Hospital, Tianjin 300380, People's Republic of China*

(Submitted 10 October 2022 – Final revision received 18 January 2023 – Accepted 31 January 2023 – First published online 9 February 2023)

Abstract

This study aimed to explore the mediation effects of one-carbon metabolism (OCM) related nutrients on the association between *MTHFR* rs1801133 polymorphism and gestational diabetes mellitus (GDM). Folate, vitamin B_{12} and homocysteine (Hcy) were measured in the serum of 1254 pregnant women. Linear and logistic regressions were used to estimate the associations of OCM nutrients and *MTHFR* rs1801133 polymorphism with blood glucose levels and GDM risk. Mediation analysis was applied to test the mediation effects of folate, vitamin B_{12} and Hcy on the association of *MTHFR* rs1801133 polymorphism with blood glucose concentrations and GDM. Pregnant women with *MTHFR* rs1801133 CC genotype had higher serum folate (10·75 *v.* 8·90 and 9·40 ng/ml) and lower serum Hcy (4·84 *v.* 4·93 and 5·20 μmol/l) than those with CT and TT genotypes. Folate concentrations were positively associated with fasting plasma glucose (FPG), 1-h plasma glucose (1-h PG), 2-h plasma glucose (2-h PG) and GDM risk. Vitamin B_{12} levels were negatively correlated with FPG and GDM. Although no direct association was found between *MTHFR* rs1801133 genotypes and GDM, there were significant indirect effects of *MTHFR* rs1801133 CC genotype on FPG (β: 0·005; 95 % CI: 0·001, 0·013), 1-h PG (β: 0·006; 95 % CI: 0·001, 0·014), 2-h PG (β: 0·007; 95 % CI: 0·001, 0·015) and GDM (β: 0·006; 95 % CI: 0·001, 0·014) via folate. In conclusion, serum folate mediates the effect of *MTHFR* rs1801133 on blood glucose levels and GDM. Our findings potentially provide a feasible GDM prevention strategy via individualised folate supplementation according to the *MTHFR* genotypes.

Key words: **Gestational diabetes mellitus: *MTHFR*: Folate: Mediation effect**

Gestational diabetes mellitus (GDM) is one of the most common pregnancy complications. The global prevalence of GDM was 14·0 %[(1)]. GDM is related to perinatal diseases and is closely related to adverse maternal and children's health after pregnancy[(2)]. Therefore, early identification of GDM-related risk factors is essential for preventing GDM and improving maternal and children's health. In addition to some traditional risk factors, increasing evidence indicates that one-carbon metabolism (OCM) related nutrients (folate and vitamin B_{12}) are associated with GDM[(3–7)].

Multiple prospective cohort studies have shown that excessive folate supplementation and higher blood folate levels are

Abbreviations: 1-h PG, 1-h plasma glucose; 2-h PG, 2-h plasma glucose; FA, folic acid; FPG, fasting plasma glucose; GDM, gestational diabetes mellitus; Hcy, homocysteine; *MTHFR*, methylenetetrahydrofolate reductase; NTD, neural tube defects; OCM, one-carbon metabolism; THF, tetrahydrofolate.

* **Corresponding author:** Qiang Zhang, email qiangzhang@tmu.edu.cn

† These authors contributed equally to this work and they shared co-first authorship.

https://doi.org/10.1017/S0007114523000314 Published online by Cambridge University Press

associated with a higher incidence of GDM[8,9]. In contrast to folate, maternal vitamin B_{12} levels were shown to be negatively correlated with GDM risk[3–6]. Since folate and vitamin B_{12} are interrelated in the OCM pathway, several studies have shown that higher folate coupled with lower vitamin B_{12} was associated with a higher risk of GDM[3,4]. Furthermore, homocysteine (Hcy) is a sensitive marker of folate and vitamin B_{12} insufficiency. However, the association between Hcy and GDM remains controversial[10].

Folate and vitamin B_{12} play a crucial role in the OCM pathway, which is driven by the folate and methionine cycles[11]. The folate cycle begins with the conversion of dietary folate into dihydrofolate, which is subsequently reduced to tetrahydrofolate (THF). THF is next converted to 5,10-methylene-THF, which is then reduced to 5-methyl-THF by methylenetetrahydrofolate reductase (*MTHFR*). As part of the methionine cycle, 5-methyl-THF donates a methyl group to regenerate methionine and THF from Hcy by the vitamin B_{12}-dependent methionine synthase (*MTR*) and methionine synthase reductase (*MTRR*). In addition to food and dietary supplements intake, serum folate, vitamin B_{12} and Hcy levels are influenced by specific genetic variants in the genes encoding these enzymes, particularly two common functional polymorphisms (rs1801131 and rs1801133) within the *MTHFR* gene[12]. Therefore, the polymorphisms of genes related to the OCM pathway may also be associated with GDM. A few recent studies have explored the relationship between *MTHFR* rs1801133 SNP and GDM. However, they did not find a significant association between them[13,14]. Our previous study evaluated the association between twelve OCM-related gene polymorphisms and GDM. We found that *MTHFR* rs1801131 was an independent risk factor for GDM, and pregnant women with the homozygous wild-type *MTHFR* rs1801131 are more susceptible to OCM-related GDM. However, no significant association was identified between *MTHFR* rs1801133 SNP and GDM using the traditional statistical methods[7].

Compared with *MTHFR* rs1801131, the effect of rs1801133 gene polymorphism on serum folate and Hcy levels was more predominant. Numerous studies have revealed that individuals with the *MTHFR* rs1801133 CC genotype have significantly higher folate levels and lower Hcy levels in serum than those with the CT and TT genotypes[15,16]. Considering folate is well associated with the risk of GDM, we speculate that the relationship between rs1801133 polymorphism and GDM is hidden or suppressed by OCM nutrients. Coincidentally, mediation analysis can contribute to a better understanding of the relationship between an independent and dependent variable through a mediator variable when these variables do not have an apparent direct association[17]. Therefore, the present study applied the mediation analysis to clarify the relationship between *MTHFR* rs1801133 polymorphism and GDM through folate, vitamin B_{12} and Hcy, respectively.

Methods

Study population

Details of the study design have been described in our previous study[7]. Briefly, pregnant women participating in this research were recruited in the Gene-Environment-Nutrient–Epigenetics interaction on Maternal and Children health study (GENEMaC) from October 2017 to September 2018 in Tianjin, China. Of the 1505 participants, 1254 pregnant women were involved in the final analysis according to the study flowchart (online Supplementary Fig. 1). The Ethics Committee of Metabolic Diseases Hospital and Institute of Endocrinology, Tianjin Medical University and Tianjin Xiqing Hospital approved the research. All procedures involving human participants were performed under the ethical standards of the Declaration of Helsinki Ethical Principles. All participants provided signed informed consent before participating in this study.

Sample collection and covariates assessment

Fasting blood samples were obtained from each pregnant woman at 24–28 gestational weeks when they underwent a routine screening for GDM. Aliquots of blood samples and serum were frozen and stored at –80 °C until further use. Information on age, education, smoking and drinking habits, parity and family history of diabetes were collected by a structured questionnaire at study enrolment. Participants' height, current weight and prepregnancy weight were also obtained through questionnaires and verified in the medical system.

Diagnosis of gestational diabetes mellitus

The primary outcome of interest for this study was the prevalence of GDM. A 75-g oral glucose tolerance test (OGTT) was used to identify GDM. The cut points of glucose values of 75-g OGTT: fasting plasma glucose (FPG) 5·1 mmol/l, 1-h plasma glucose (1-h PG) 10·0 mmol/l and 2-h plasma glucose (2-h PG) 8·5 mmol/l. GDM can be diagnosed when any one value is equalled or exceeded.

Determination of one-carbon metabolism-related nutrients

The method for determining folate, vitamin B_{12} and Hcy in maternal serum has been described in detail in our previous studies[7,18]. Briefly, folate and vitamin B_{12} were measured using the Architect-i2000SR automated chemiluminescence immunoassay system and its supporting kit (Abbott Diagnostics). A circulating enzymatic method was adopted for the determination of serum Hcy by the automatic biochemical analyzer (Dirui CS-T300) and Hcy kit (MedicalSystem Biotechnology).

Genotyping of MTHFR *rs1801131 and rs1801133*

The two SNP in *MTHFR* (rs1801311 and rs1801133) were genotyped at Biowing biotechnology using the assay conditions described previously[7,19]. In brief, human genomic DNA were extracted from blood samples with RelaxGene Blood DNA System (Tiangen Biotech) following the manufacturer's instructions. After three-round multiplex PCR amplification, the products were subjected to next generation sequencing platform to genotype SNP precisely.

Statistical analysis

The Chi-square test or Fisher's exact test was applied for categorical variables to examine the differences between

British Journal of Nutrition
https://doi.org/10.1017/S0007114523000314 Published online by Cambridge University Press

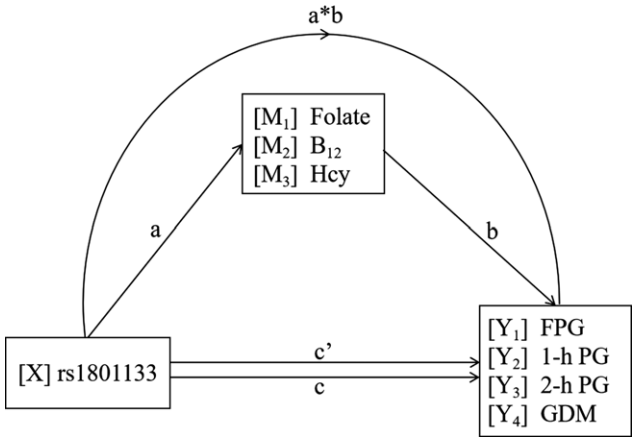

Fig. 1. Conceptual diagram of mediation analysis. a, regression coefficient of X *v.* Mi; b, regression coefficient of Mi *v.* Yi; a * b, indirect effect, regression coefficient of the mediation effect; c, total effect, regression coefficient when X *v.* Yi (no mediator variable Mi in the model); c', direct effect, regression coefficient when X *v.* Yi (mediator variable Mi in the model). All models were adjusted for age, prepregnancy BMI, education, smoking, drinking, family history of diabetes, parity and rs1801131 genotypes. Additionally, serum vitamin B_{12} and Hcy were adjusted when folate worked as mediator; folate and Hcy were adjusted when vitamin B_{12} worked as mediator; and folate and vitamin B_{12} were adjusted when Hcy worked as mediator.

different rs1801133 genotypes. For continuous variables with skewed distribution, the Kruskal–Wallis *H* test was employed to determine the differences among rs1801133 CC, CT and TT genotypes. Spearman's correlation was conducted to estimate monotonic relationships among serum folate, vitamin B_{12} and Hcy levels. Linear regression was applied to examine associations of rs1801133 genotypes and serum OCM nutrients with plasma glucose levels. Logistic regression was used to calculate OR and 95 % CI for rs1801133 genotypes and serum OCM nutrients on GDM. Potential maternal confounders, including age, prepregnancy BMI, education, smoking, drinking, family history of diabetes, parity and rs1801131 genotypes, were adjusted in all models. In addition, serum OCM nutrients (folate, vitamin B_{12} and Hcy) were mutually adjusted to evaluate the association of OCM nutrients with blood glucose levels and GDM. When estimating the association of rs1801133 genotypes with blood glucose levels and GDM, maternal confounders and serum OCM nutrients were adjusted.

The best inheritance model for *MTHFR* rs1801133 on GDM was the dominant model based on the smallest value of the Akaike Information Criterion and Bayesian Information Criterion (see the Results section). Therefore, the mediating effects of OCM nutrients on the relationship between rs1801133 genotypes and blood glucose levels (FPG, 1-h PG and 2-h PG) and GDM were examined based on the dominant model (Fig. 1). According to the recommendation by Baron and Kenny[20], the mediation model included the following steps: (1) the linear or logistic regression model estimated the association of rs1801133 genotypes with blood glucose levels and GDM without adjusting for OCM nutrients; (2) the relationship between rs1801133 genotypes and OCM nutrients was evaluated by a linear regression model (a path); (3) the association between OCM nutrients and GDM (b path) was analysed using a logistic regression model; (4) the OCM mediator (folate, vitamin B_{12} and Hcy) was added to the c path. If the path between rs1801133 genotypes and GDM was no longer significant, it could be concluded that the association between rs1801133 genotypes and GDM was fully mediated by OCM nutrients (c' path). Otherwise, the association might be only partially mediated by OCM nutrients.

All statistical analyses were performed using R (version 4.1.2; R Project for Statistical Computing). Mediation analysis was implemented with the R package 'bruceR'. A *P*-value < 0·05 was considered to be statistically significant.

Results

General information on the study population

The genotype frequencies for the *MTHFR* rs1801133 were in Hardy–Weinberg equilibrium. Genotype distribution of the rs1801133 SNP was 19·14 %, 48·64 % and 32·22 % for the CC, CT and TT genotypes, respectively (online Supplementary Table 1). There were no significant differences in demographic characteristics among different *MTHFR* genotypes. However, there were significant differences in folate (CC: 10·75 ng/ml, CT: 8·90 ng/ml and TT: 9·40 ng/ml) and Hcy (CC: 4·84 μmol/l, CT: 4·93 μmol/l and TT: 5·20 μmol/l) levels among the three groups. In addition, pregnant women with the rs1801133 CT and TT genotypes were more likely to have the rs1801131 TT genotype. The three groups observed no significant differences in blood glucose levels and GDM prevalence (Table 1). The correlations of serum folate and vitamin B_{12} concentrations with Hcy levels are displayed in Supplementary Fig. 2. Serum folate was positively correlated with vitamin B_{12}, and serum Hcy levels were negatively correlated with folate and vitamin B_{12} concentrations.

Associations of one-carbon metabolism indicators with blood glucose and gestational diabetes mellitus

Folate concentrations were positively associated with blood glucose concentrations in both crude and adjusted models, especially for 1-h PG and 2-h PG. However, serum vitamin B_{12} levels were negatively correlated with FPG concentrations in crude and adjusted models. There was no significant association between serum vitamin B_{12} and 1-h PG and 2-h PG. In contrast to folate, serum Hcy levels had a negative correlation with 2-h PG concentrations in crude (β: −0·08; 95 % CI: −0·13, −0·04) and adjusted (β: −0·06; 95 % CI: −0·10, −0·01) models (Table 2). The association between OCM indicators and GDM can be found in Fig. 2. Serum folate was positively related to GDM. However, vitamin B_{12} was negatively related to GDM. There was no significant association between Hcy and GDM risk.

Association of MTHFR *rs1801133 polymorphism with blood glucose and gestational diabetes mellitus*

There were no substantial differences regarding the blood glucose levels of the increase of the T allele in rs1801133 reflected by the beta values (online Supplementary Table 2). Compared with pregnant women with the CC genotype, participants with CT (OR: 1·34; 95 % CI: 0·86, 2·07) and TT (OR: 0·99;

British Journal of Nutrition

https://doi.org/10.1017/S0007114523000314 Published online by Cambridge University Press

Table 1. Characteristics of the study population according to *MTHFR* rs1801133 genotypes (*n* 1254)

Characteristics	CC (*n* 240)		CT (*n* 610)		TT (*n* 404)		*P*-values
	n	%	*n*	%	*n*	%	
Demographics							
Age (years)							
Median	29		30		30		0·515
25th and 75th percentiles	26–33		27–33		27–32		
Prepregnancy BMI (kg/m^2)	22·31	20·34–25·11	22·68	20·55–25·27	22·57	20·31–25·22	0·558
Education (years)							0·842
≤ 12	104	43·33	240	39·34	166	41·09	
12~15	72	30·00	197	32·30	131	32·43	
> 15	64	26·67	173	28·36	107	26·49	
Smoking							0·393
No	238	99·17	603	98·85	396	98·02	
Yes	2	0·83	7	1·15	8	1·98	
Drinking							0·729
No	239	99·58	605	99·18	400	99·01	
Yes	1	0·42	5	0·82	4	0·99	
Family history of diabetes							0·467
No	224	93·33	556	91·15	366	90·59	
Yes	16	6·67	54 (8·85)8		38	9·41	
Parity							0·538
Nulliparous	113	47·08	308	50·49	192	47·52	
Multiparous	127	52·92	302	49·51	212	52·48	
OCM indicators							
Folate (ng/ml)							
Median	10·75		8·90		9·40		0·010
25th and 75th percentiles	7·10–15·03		6·20–13·90		5·43–14·80		
Vitamin B_{12} (pg/ml)							
Median	279		266		272		0·168
25th and 75th percentiles	221–357		210–336		211–335		
Hcy (μmol/l)							
Median	4·84		4·93		5·20		<0·001
25th and 75th percentiles	4·30–5·66		4·47–5·85		4·57–6·87		
rs1801131							<0·001
TT	107	44·58	406	66·56	404	100	
TG/GG	133	55·42	204	33·44	0	0	
Blood glucose levels (mmol/l)							
FPG							
Median	4·65		4·63		4·64		0·853
25th and 75th percentiles	4·43–4·88		4·40–4·92		4·41–4·92		
1-h PG							
Median	7·30		7·28		7·33		0·795
25th and 75th percentiles	6·50–8·46		6·30–8·47		6·46–8·42		
2-h PG							
Median	6·47		6·44		6·47		0·803
25th and 75th percentiles	5·86–7·13		5·73–7·31		5·68–7·18		
GDM status							0·160
No	203	84·58	481	78·85	322	79·70	
Yes	37	15·42	129	21·15	82	20·30	

OCM, one-carbon metabolism; FPG, fasting plasma glucose; GDM, gestational diabetes mellitus.
Values are *n* (%) or median (25th and 75th percentiles). *P*-values were obtained by Krushkal–Wallis *H* test for continuous variables and Chi-square test or Fisher's exact test for categorical variables.

95 % CI: 0·60, 1·62) genotypes did not have a significantly higher risk of GDM (Table 3). Similarly, there were no significant associations between *MTHFR* rs1801133 SNP and GDM in the dominant, recessive, over-dominant and additive models (Table 3).

Mediation of one-carbon metabolism indicators on the association of MTHFR *rs1801133 genotype with blood glucose and gestational diabetes mellitus*

The mediation effects of serum folate on the association of *MTHFR* rs1801133 genotype with FPG, 1-h PG, 2-h PG and GDM are shown in Fig. 3. Using the CT/TT genotypes as the reference, the total effect model predicting FPG revealed a non-significant effect of *MTHFR* rs1801133 CC genotype after adjustment for maternal characteristics, rs1801131 genotypes and serum vitamin B_{12} and Hcy levels. Once folate was added to the model, the direct effect of the *MTHFR* rs1801133 CC genotype on FPG was also insignificant (Fig. 3(a)). However, there was a significant indirect effect of the *MTHFR* rs1801133 CC genotype on FPG. The regression coefficient of the mediation effect was 0·005 (95 % CI: 0·001, 0·013), indicating that folate plays a mediating role in the effect of *MTHFR* rs1801133 on FPG (Fig. 3(a)). Similarly, the indirect effects of the *MTHFR* rs1801133 CC genotype on 1-h PG (β: 0·006; 95 % CI: 0·001, 0·014), 2-h PG (β: 0·007; 95 % CI: 0·001, 0·015) and GDM (β: 0·006; 95 % CI: 0·001, 0·014) were significant (Fig. 3(b),

British Journal of Nutrition
https://doi.org/10.1017/S0007114523000314 Published online by Cambridge University Press

Table 2. Association of maternal serum folate, vitamin B_{12} and Hcy levels with FPG, 1-h PG and 2-h PG (*n* 1254)

	FPG			1-h PG			2-h PG		
OCM indicators	*β*	95 % CI	*P*-values	*β*	95 % CI	*P*-values	*β*	95 % CI	*P*-values
Per IQR increase in folate									
Crude model	0·05	0·01, 0·09	0·023	0·44	0·29 0·59	<0·001	0·35	0·23, 0·47	<0·001
Adjusted Model*	0·07	0·03, 0·12	0·002	0·32	0·16, 0·48	<0·001	0·29	0·15, 0·42	<0·001
Per IQR increase in vitamin B_{12}									
Crude model	−0·04	−0·07, −0·02	<0·001	0·07	−0·03, 0·16	0·156	0·04	−0·03, 0·12	0·270
Adjusted Model*	−0·05	−0·08, −0·03	<0·001	−0·01	−0·11, 0·09	0·829	−0·04	−0·12, 0·04	0·343
Per IQR increase in Hcy									
Crude model	−0·00	−0·02, 0·02	0·951	−0·07	−0·13, −0·01	0·024	−0·08	−0·13, −0·04	<0·001
Adjusted Model*	0·00	−0·02, 0·02	0·932	−0·02	−0·08, 0·04	0·442	−0·06	−0·10, −0·01	0·022

Hcy, homocysteine; FPG, fasting plasma glucose; 1-h PG, 1-h plasma glucose; 2-h PG, 2-h plasma glucose; OCM, one-carbon metabolism.
* Adjusted for age, prepregnancy BMI, education, smoking, drinking, family history of diabetes, parity and rs1801131 genotypes. Additionally, serum vitamin B_{12} and Hcy were adjusted in the folate group; folate and Hcy were adjusted in the vitamin B_{12} group; and folate and vitamin B_{12} were adjusted in the Hcy group.

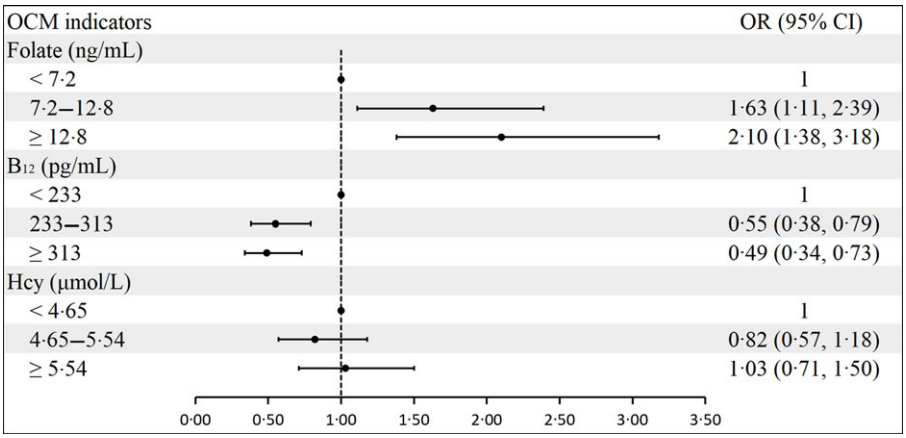

Fig. 2. Association of maternal serum folate, vitamin B_{12} and Hcy levels with GDM. [a] Adjusted for age, prepregnancy BMI, education, smoking, drinking, family history of diabetes, parity and rs1801131 genotypes. Additionally, serum vitamin B_{12} and Hcy were adjusted in the folate group; folate and Hcy were adjusted in the vitamin B_{12} group; and folate and vitamin B_{12} were adjusted in the Hcy group. GDM, gestational diabetes mellitus.

Table 3. Odds ratios of *MTHFR* rs1801133 genotype for GDM (*n* 1254)

Genetic models	GDM	Non-GDM	OR	95 % CI*	*P*-values	OR	95 % CI†	*P*-values	AIC	BIC
Co-dominant model										
CC	37	203	1			1				
CT	129	481	1·47	0·99, 2·20	0·059	1·34	0·86, 2·07	0·194	1249·4	1264·8
TT	82	322	1·40	0·91, 2·14	0·124	0·99	0·60, 1·62	0·960		
Dominant model										
CC	37	203	1			1				
CT/TT	211	803	1·44	0·98, 2·11	0·060	1·23	0·80, 1·88	0·352	1247·5	1257·7
Recessive model										
CC/CT	166	684	1			1				
TT	82	322	1·05	0·78, 1·41	0·750	0·78	0·56, 1·10	0·153	1251·1	1261·4
Over-dominant model										
CC/TT	119	525	1			1				
CT	129	481	1·18	0·90, 1·56	0·236	1·35	0·99, 1·82	0·052	1249·8	1260·1
Additive model										
CC/CT/TT	248	1006	1·14	0·93, 1·39	0·206	0·95	0·75, 1·19	0·640	1249·6	1259·9

GDM, gestational diabetes mellitus; AIC, Akaike Information Criterion; BIC, Bayesian Information Criterion; Hcy, homocysteine.
* Crude model.
† Adjusted for age, prepregnancy BMI, education, smoking, drinking, family history of diabetes, parity, serum folate, vitamin B_{12}, Hcy and rs1801131 genotypes.

(c) and (d)). The regression coefficients of the path 'a' (rs1801133 CC genotype *v.* folate) and path 'b' (folate *v.* blood glucose levels and GDM) indicate that *MTHFR* rs1801133 CC genotype indirectly predicted higher blood glucose levels or higher GDM risk by increasing serum folate concentrations. No significant indirect effect was found between the *MTHFR* rs1801133 CC genotype and FPG, 1-h PG, 2-h PG and GDM when vitamin B_{12} worked as mediators (online Supplementary Fig. 3). However, we did

British Journal of Nutrition
https://doi.org/10.1017/S0007114523000314 Published online by Cambridge University Press

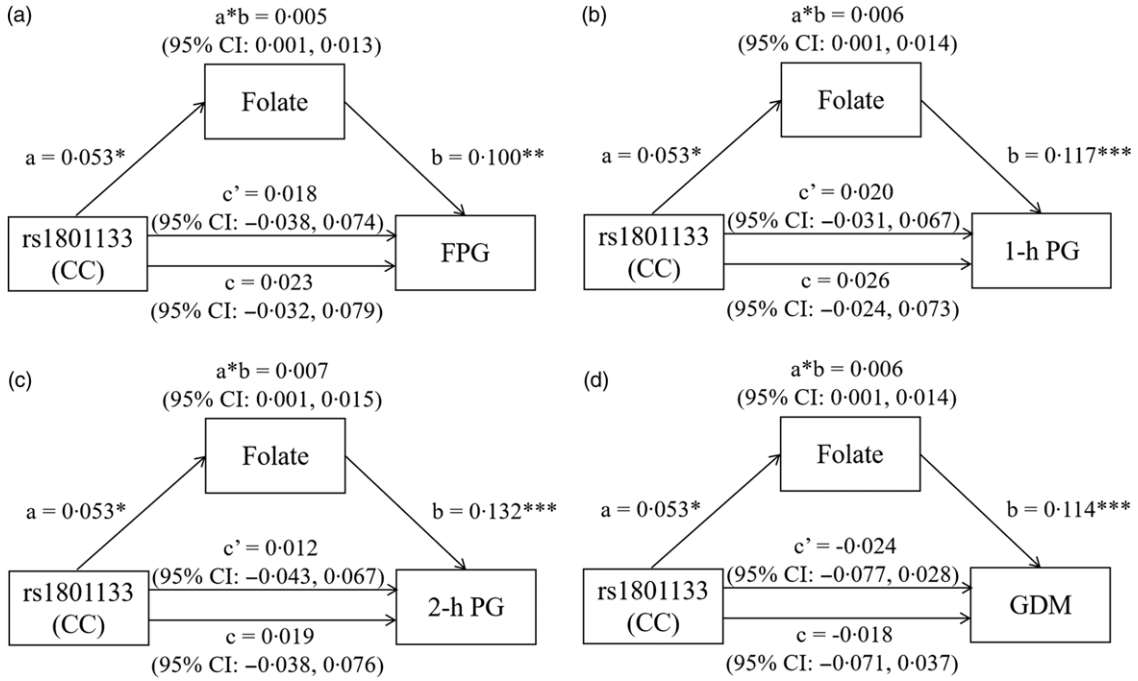

Fig. 3. The mediation effect of folate on the association of *MTHFR* rs1801133 with (a) FPG, (b) 1-h PG, (c) 2-h PG and (d) GDM. All models were adjusted for age, prepregnancy BMI, education, smoking, drinking, family history of diabetes, parity, rs1801131 genotypes, serum vitamin B_{12} and Hcy. a, regression coefficient of rs1801133 *v.* folate; b, regression coefficient of folate *v.* FPG, 1-h PG, 2-h PG and GDM; a * b, indirect effect, regression coefficient of the mediation effect; c, total effect, regression coefficient when rs1801133 *v.* FPG, 1-h PG, 2-h PG and GDM (no mediating variable folate in the model); c', direct effect, regression coefficient when rs1801133 *v.* FPG, 1-h PG, 2-h PG and GDM (mediating variable folate in the model). * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$. FPG, fasting plasma glucose; 1-h PG, 1-h plasma glucose; 2-h PG, 2-h plasma glucose; GDM, gestational diabetes mellitus.

observe a significant indirect effect of wild-type *MTHFR* rs1801133 (β: 0·004; 95 % CI: 0·001, 0·009), such that the CC genotype predicted higher 2-h PG indirectly through decreasing serum Hcy levels (online Supplementary Fig. 4C).

Discussion

In this study, we evaluated the mediation effect of serum OCM nutrients on the association between *MTHFR* rs1801133 polymorphism and GDM in Chinese Han pregnant women for the first time. Although there was no significant association between *MTHFR* rs1801133 genotypes and GDM risk, we found a novel and significant indirect effect of *MTHFR* rs1801133 polymorphism on blood glucose levels and GDM through modifying serum folate levels.

Folate and vitamin B_{12} are essential water-soluble vitamins. In addition to food and dietary supplementation, genetic polymorphisms in *MTHFR*, *MTR* and *MTRR* are closely related to serum folate, vitamin B_{12} and Hcy levels[12,21]. Among these genes, rs1801133 in *MTHFR* is the most well-known genetic factor influencing OCM status. It was reported that carriers of the rs1801133 T allele have lower enzyme activity, which leads to decreased folate and increased Hcy concentrations[22]. In contrast, the rs1801133 CC genotype is associated with higher folate and lower Hcy. In the present study, the prevalence of the rs1801133 CC, CT and TT genotypes were 19·14 %, 48·64 % and 32·22 %, respectively. This was in line with previously reported CC, CT and TT frequencies in Tianjin, China (21·4 %, 48·3 % and 30·3 %, respectively)[23]. We found that pregnant women with the rs1801133 CC genotype have higher folate and lower Hcy concentrations. These results are consistent with previous findings that the wide-type (CC) of *MTHFR* rs1801133 was associated with increased folate but decreased Hcy levels[12]. Furthermore, there was no significant difference in vitamin B_{12} levels among rs1801133 CC, CT and TT genotypes. Our findings were in line with most previous studies that genetic polymorphisms in *MTHFR* (rs1801131 and rs1801133) have no association with vitamin B_{12} concentrations[24]. However, a study from Jordan indicated that the rs1801133 T allele was associated with vitamin B_{12} deficiency[25]. In addition to ethnic differences, it could be postulated that the rs1801133 polymorphism might be related to decreased intestinal absorption of vitamin B_{12}[26].

Although the relationship between high folate and low vitamin B_{12} and GDM is well studied, their association with blood glucose is limited. A prospective cohort study from the UK revealed that folate was positively associated with 2-h PG, and vitamin B_{12} was negatively associated with FPG and 2-h PG. However, they did not provide the data of 1-h PG[5]. Our study indicated that serum folate was positively associated with blood glucose levels. However, this association was more evident in 1-h PG and 2-h PG. For vitamin B_{12}, a significantly positive correlation was found only for FPG. Although the association between Hcy and GDM was insignificant, it was negatively correlated with 2-h PG. This contrasts sharply with the relationship between folate and blood glucose levels since Hcy is a surrogate marker of folate deficiency. Our findings were

British Journal of Nutrition

https://doi.org/10.1017/S0007114523000314 Published online by Cambridge University Press

in line with recently published results, which indicated that Hcy is inversely associated with blood glucose levels[3]. However, other studies revealed that the relationship between Hcy and GDM remains controversial[10,27]. Therefore, a comprehensive evaluation of the factors related to Hcy metabolism may help clarify the association between Hcy and GDM.

Since the relationship between folate, vitamin B_{12} and GDM is significant, and genetic variants in *MTHFR* can significantly affect the OCM nutrient status, we speculate that SNPs in this gene may be closely related to blood glucose levels and GDM risk. However, we did not observe a significant association between *MTHFR* rs1801133 SNP and GDM under different genetic models. Similarly, we did not find a significant association between mutant T and blood glucose levels at different time points of OGTT (online Supplementary Table 2). The research on the relationship between *MTHFR* rs1801133 SNP and GDM is still limited. Studies from India, Australia and South Africa showed that the maternal *MTHFR* rs1801133 genotype was not associated with GDM risk[13,28,29]. Similar results were also found in the Chinese population[14]. Our findings and others indicated that the relationship between *MTHFR* rs1801133 and GDM may not be direct. This also urged us to consider new and more sensitive methods to reveal the relationship between *MTHFR* rs1801133 and GDM.

The *MTHFR* rs1801133 polymorphism affects serum OCM nutrient levels, and folate, vitamin B_{12} and Hcy affect blood glucose levels and GDM occurrence. It is reasonable to raise the question of whether a genetic variant in *MTHFR* rs1801133 will affect blood glucose levels and GDM occurrence by influencing OCM nutrient levels. A question like that suggests a chain of relations where an independent variable (X) affects a mediating variable (M), which then affects a dependent variable (Y). Therefore, the mediation analysis is the best way to determine the effect of M transmits the effect of X on Y[30,31]. Consistent with our hypothesis, serum folate mediated the effect of the *MTHFR* rs1801133 on FPG, 1-h PG, 2-h PG and GDM. The regression coefficients of the *MTHFR* rs1801133 polymorphism with folate and folate with blood glucose levels and GDM indicated that the *MTHFR* rs1801133 CC genotype predicts higher blood glucose levels or evaluated GDM risk indirectly through increasing serum folate concentrations. However, the mediating role of vitamin B_{12} in transmitting the effect of the *MTHFR* rs1801133 polymorphism on blood glucose levels and GDM was insignificant. This may be attributed to the fact that the effect of the *MTHFR* rs1801133 polymorphism on serum vitamin B_{12} levels is not apparent. Although no significant indirect effect was found between *MTHFR* rs1801133 polymorphism and GDM when Hcy served as a mediating variable, the mediation effect of Hcy in transmitting the effect between *MTHFR* rs1801133 polymorphism on 2-h PG was significant (online Supplementary Fig. 4(c)). Although this is the first study on the relationship between *MTHFR* and GDM by mediation analysis, a few studies have focussed on the association between genetic polymorphisms and metabolic disease through this analysis approach[32,33]. It highlights that mediation analysis is a powerful method to test a hypothetical causal chain where one variable affects a second variable and, in turn, that variable affects the third variable.

The mechanisms linking high folate and low vitamin B_{12} levels and increased GDM risk remain unclear. However, serum folate mediated the effect of *MTHFR* rs1801133 SNP on GDM, which provided a biological pathway for understanding how the *MTHFR* polymorphism affects GDM during pregnancy. Our previous study revealed that the wild-type (TT) genotype of *MTHFR* rs1801131 was significantly associated with an increased risk of GDM. In addition, pregnant women with homozygous T alleles are more prone to have OCM-related GDM[7]. However, the present study showed an indirect effect of *MTHFR* rs1801133 SNP on GDM by modifying serum folate levels using the mediation approach. The two studies all pointed out that the wild-type genotypes of *MTHFR* rs1801131 and rs1801133 with higher folate can increase the risk of GDM but through different ways, as rs1801131 acting directly and rs1801133 indirectly. In the OCM pathway, Hcy can be remethylated to methionine via vitamin B_{12}-dependent *MTR* and *MTRR* utilising the methyl group from 5-methyl-THF. Without vitamin B_{12}, 5-methyl-THF becomes metabolically trapped in this form since producing 5-methyl-THF from 5, 10-methylene-THF by *MTHFR* is irreversible[34]. In addition, the wild-type genotypes of *MTHFR* rs1801133 and rs1801131 facilitate reducing 5, 10-methylene-THF to 5-methyl-THF. The above evidence indicates that folate/vitaminB_{12} imbalance coupled with pregnant women's *MTHFR* rs1801133 CC and rs1801131 TT genotype is more likely to accumulate 5-methyl-THF in the body. Although the role of 5-methyl-THF in the pathogenesis of GDM is unclear, recent animal studies suggested that consuming 5-methyl-THF diets will increase the weight of the dams and their female offspring[35,36]. Therefore, 5-methyl-THF may play a role in GDM occurrence, and further studies are needed to clarify the underlying mechanism.

Folic acid (FA) supplementation in the periconceptional period is recommended to prevent neural tube defects (NTD) worldwide. Some countries, including the USA and Canada, have implemented mandatory fortification of cereal grains with FA to prevent NTD. Although there is no mandatory FA fortification in China, a nationwide FA supplement project has been launched since 2009 with a recommendation of 400 μg/d of folate supplementation from 3 months of preconception until 3 months of pregnancy. A recent study in Tianjin showed that 93·1 % of pregnant women took FA supplements before and/or during pregnancy. However, only 14·4 % of them adhered to the recommendation for FA intake[37]. It was reported that 42·3 % of the pregnant women took a FA dose exceeding 400 μg/d, and 69·5 % took FA longer than the recommended duration[37]. The indiscriminately FA intake may lead to significantly higher folate levels among pregnant women, especially those with wild-type genotypes of *MTHFR*, which may account for the high incidence of GDM. However, for pregnant women carrying the mutant allele of *MTHFR*, higher folate intake may not cause GDM but is beneficial for NTD prevention since the mutant genotypes of *MTHFR* are associated with lower folate concentrations and are more likely to affect NTD in the offspring. Our findings do not conflict with the current strategy of FA supplementation to prevent NTD, but for *MTHFR* wild-type genotype carriers, high-dose FA supplementation should be avoided.

The strengths of this study include the availability of individual measurements of serum folate, vitamin B_{12} and Hcy

British Journal of Nutrition

https://doi.org/10.1017/S0007114523000314 Published online by Cambridge University Press

levels, the ability to estimate the mediation effect of folate on the association between *MTHFR* rs1801133 polymorphism and GDM, and larger sample sizes that give more reliable results. Despite these advantages, our study was subject to some limitations. First, due to the nature of the cross-sectional design, the temporal link between OCM nutrients and GDM cannot be determined. However, the mediation analysis can estimate how much *MTHFR* rs1801133 polymorphism may affect GDM through a potential causal mechanism by modifying folate levels. Second, serum folate levels are closely related to folate intake and *MTHFR* genotype. The lack of data on folate and vitamin B_{12} from food and dietary supplement limited the application of our findings. Third, folate species, especially 5-methyl-THF, were not distinguished. Further studies are required to verify the potential role of 5-methyl-THF in GDM.

In conclusion, maternal *MTHFR* rs1801133 polymorphism was not directly associated with GDM risk in Chinese Han pregnant women. However, serum folate can mediate their association indirectly. To our knowledge, this is the first epidemiologic study to use a mediation approach to evaluate the indirect effect of OCM-related gene polymorphisms on GDM through modifying folate, vitamin B_{12} and Hcy levels. Further mechanistic studies are encouraged to figure out the potential role of OCM nutrients in GDM. At the same time, a more accurate assessment of folate and vitamin B_{12} supplementation in pregnant women with different genotypes should also be conducted. Our findings provide a feasible GDM prevention strategy via precision nutrition in the future.

Acknowledgements

All the authors would like to thank the participants who took part in this study.

This work was supported by the Fundamental Research Funds for Higher Education of Tianjin Municipal Education Commission (2020ZD14).

Q. Z. and X. Z. conceived and designed the research; Y. W. (Yunguo Wang), Y. S., S. L. (Shuying Li), L. D., C. S. and Y. Y. conducted the research; Y. W. (Yiyun Wang), X. L. and S. L. (Suhui Luo) contributed to laboratory sample testing; Y. W. (Yiyun Wang) and N. Z. performed the statistical analyses; Y. W. (Yunguo Wang) and Q. Z. wrote the manuscript; Q. Z. had primary responsibility for the final content; and all authors contributed to the interpretation of the results and approved the final version of the manuscript.

The authors declare that they have no conflicts of interest.

Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114523000314

References

1. Wang H, Li N, Chivese T, *et al.* (2022) IDF diabetes atlas: estimation of global and regional gestational diabetes mellitus prevalence for 2021 by International Association of Diabetes in Pregnancy Study Group's Criteria. *Diabetes Res Clin Pract* **183**, 109050.
2. McIntyre HD, Catalano P, Zhang C, *et al.* (2019) Gestational diabetes mellitus. *Nat Rev Dis Primers* **5**, 47.
3. Lai JS, Pang WW, Cai S, *et al.* (2018) High folate and low vitamin B_{12} status during pregnancy is associated with gestational diabetes mellitus. *Clin Nutr* **37**, 940–947.
4. Li S, Hou Y, Yan X, *et al.* (2019) Joint effects of folate and vitamin B_{12} imbalance with maternal characteristics on gestational diabetes mellitus. *J Diabetes* **11**, 744–751.
5. Saravanan P, Sukumar N, Adaikalakoteswari A, *et al.* (2021) Association of maternal vitamin B_{12} and folate levels in early pregnancy with gestational diabetes: a prospective UK cohort study (PRiDE study). *Diabetologia* **64**, 2170–2182.
6. Chen X, Zhang Y, Chen H, *et al.* (2021) Association of maternal folate and vitamin B_{12} in early pregnancy with gestational diabetes mellitus: a prospective cohort study. *Diabetes Care* **44**, 217–223.
7. Li S, Tian X, Wang Y, *et al.* (2022) Associations of maternal rs1801131 genotype in MTHFR and serum folate and vitamin B_{12} with gestational diabetes mellitus in Chinese pregnant women. *Nutrients* **14**, 1169.
8. Zhu B, Ge X, Huang K, *et al.* (2016) Folic acid supplement intake in early pregnancy increases risk of gestational diabetes mellitus: evidence from a prospective cohort study. *Diabetes Care* **39**, e36–e37.
9. Li Q, Zhang Y, Huang L, *et al.* (2019) High-dose folic acid supplement use from prepregnancy through midpregnancy is associated with increased risk of gestational diabetes mellitus: a prospective cohort study. *Diabetes Care* **42**, e113–e115.
10. Zheng Y, Deng H-Y, Qiao Z-Y, *et al.* (2021) Homocysteine level and gestational diabetes mellitus: a systematic review and meta-analysis. *Gynecol Endocrinol* **37**, 1–8.
11. Clare CE, Brassington AH, Kwong WY, *et al.* (2019) One-carbon metabolism: linking nutritional biochemistry to epigenetic programming of long-term development. *Annu Rev Anim Biosci* **7**, 263–287.
12. Hiraoka M & Kagawa Y (2017) Genetic polymorphisms and folate status. *Congenit Anom* **57**, 142–149.
13. Khan IA, Shaik NA, Kamineni V, *et al.* (2015) Evaluation of gestational diabetes mellitus risk in south Indian women based on MTHFR (C677T) and FVL (G1691A) mutations. *Front Pediatr* **3**, 34.
14. Liu PJ, Liu Y, Ma L, *et al.* (2020) Associations between gestational diabetes mellitus risk and folate status in early pregnancy and MTHFR C677T polymorphisms in Chinese women. *Diabetes Metab Syndr Obes* **13**, 1499–1507.
15. Shane B, Pangilinan F, Mills JL, *et al.* (2018) The 677C→T variant of MTHFR is the major genetic modifier of biomarkers of folate status in a young, healthy Irish population. *Am J Clin Nutr* **108**, 1334–1341.
16. Jin H, Cheng H, Chen W, *et al.* (2018) An evidence-based approach to globally assess the covariate-dependent effect of the MTHFR single nucleotide polymorphism rs1801133 on blood homocysteine: a systematic review and meta-analysis. *Am J Clin Nutr* **107**, 817–825.
17. MacKinnon DP, Fairchild AJ & Fritz MS (2007) Mediation analysis. *Annu Rev Psychol* **58**:593–614.
18. Zhang Q, Zhang X, Li S, *et al.* (2021) Joint effect of urinary arsenic species and serum one-carbon metabolism nutrients on gestational diabetes mellitus: a cross-sectional study of Chinese pregnant women. *Environ Int* **156**, 106741.
19. Chen K, Zhou Y-X, Li K, *et al.* (2016) A novel three-round multiplex PCR for SNP genotyping with next generation sequencing. *Anal Bioanal Chem* **408**, 4371–4377.

British Journal of Nutrition

https://doi.org/10.1017/S0007114523000314 Published online by Cambridge University Press

20. Baron RM & Kenny DA (1986) The moderator-mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. *J Pers Soc Psychol* **51**, 1173–1182.
21. Thuesen BH, Husemoen LLN, Ovesen L, *et al.* (2010) Lifestyle and genetic determinants of folate and vitamin B_{12} levels in a general adult population. *Br J Nutr* **103**, 1195–1204.
22. Tsang BL, Devine OJ, Cordero AM, *et al.* (2015) Assessing the association between the methylenetetrahydrofolate reductase (MTHFR) 677C > T polymorphism and blood folate concentrations: a systematic review and meta-analysis of trials and observational studies. *Am J Clin Nutr* **101**, 1286–1294.
23. Yang B, Liu Y, Li Y, *et al.* (2013) Geographical distribution of MTHFR C677T, A1298C and MTRR A66G gene polymorphisms in China: findings from 15357 adults of Han nationality. *PLoS One* **8**, e57917.
24. Surendran S, Adaikalakoteswari A, Saravanan P, *et al.* (2018) An update on vitamin B_{12}-related gene polymorphisms and B_{12} status. *Genes Nutr* **13**, 2.
25. Al-Batayneh KM, Zoubi MSA, Shehab M, *et al.* (2018) Association between MTHFR 677C > T polymorphism and vitamin B_{12} deficiency: a case-control study. *J Med Biochem* **37**, 141–147.
26. Shiran A, Remer E, Asmer I, *et al.* (2015) Association of vitamin B_{12} deficiency with homozygosity of the TT MTHFR C677T genotype, hyperhomocysteinemia, and endothelial cell dysfunction. *Isr Med Assoc J* **17**, 288–292.
27. Radzicka S, Ziolkowska K, Zaborowski MP, *et al.* (2019) Serum homocysteine and vitamin B_{12} levels in women with gestational diabetes mellitus. *Ginekol Pol* **90**, 381–387.
28. Dias S, Adam S, Rheeder P, *et al.* (2021) No association between ADIPOQ or MTHFR polymorphisms and gestational diabetes mellitus in South African women. *Diabetes Metab Syndr Obes* **14**, 791–800.
29. Jankovic-Karasoulos T, Furness DL, Leemaqz SY, *et al.* (2020) Maternal folate, one-carbon metabolism and pregnancy outcomes. *Matern Child Nutr* **17**, e13064.
30. Preacher KJ (2015) Advances in mediation analysis: a survey and synthesis of new developments. *Annu Rev Psychol* **66**, 825–852.
31. Lee H, Cashin AG, Lamb SE, *et al.* (2021) A guideline for reporting mediation analyses of randomized trials and observational studies: the AGReMA statement. *JAMA* **326**, 1045–1056.
32. Xu M, Lv X, Xie L, *et al.* (2016) Discrete associations of the GCKR variant with metabolic risk in a Chinese population: longitudinal change analysis. *Diabetologia* **59**, 307–315.
33. Guerra-García MT, Moreno-Macías H, Ochoa-Guzmán A, *et al.* (2021) The -514C > T polymorphism in the LIPC gene modifies type 2 diabetes risk through modulation of HDL-cholesterol levels in Mexicans. *J Endocrinol Invest* **44**, 557–565.
34. Maher A & Sobczyńska-Malefora A (2021) The relationship between folate, vitamin B_{12} and gestational diabetes mellitus with proposed mechanisms and foetal implications. *J Fam Reprod Health* **15**, 141–149.
35. Pannia E, Hammoud R, Simonian R, *et al.* (2020) [6S]-5-methyltetrahydrofolic acid and folic acid pregnancy diets differentially program metabolic phenotype and hypothalamic gene expression of wistar rat dams post-birth. *Nutrients* **13**, E48.
36. Pannia E, Hammoud R, Kubant R, *et al.* (2021) High intakes of [6S]-5-methyltetrahydrofolic acid compared with folic acid during pregnancy programs central and peripheral mechanisms favouring increased food intake and body weight of mature female offspring. *Nutrients* **13**, 1477.
37. Yan J, Zheng YZ, Cao LJ, *et al.* (2017) Periconceptional folic acid supplementation in Chinese women: a cross-sectional study. *Biomed Environ Sci* **30**, 737–748.

British Journal of Nutrition

https://doi.org/10.1017/S0007114523000314 Published online by Cambridge University Press