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RESEARCH PAPER

Maternal dietary intake of folate, vitamin B_{12} and *MTHFR 677C>T* genotype: their impact on newborn's anthropometric parameters

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Abstract In this study, we evaluated the effects of dietary intake of vitamin B_{12} and folate during pregnancy and their interactions with maternal polymorphism of *MTHFR* (*677C>T; 1298A>C*) on intrauterine development. Anthropometric parameters were obtained from 231 newborns that belong to a prospective birth cohort in Morelos, Mexico. Maternal dietary intake of vitamin B_{12} and folate was assessed using a semi-quantitative questionnaire administered during the first and third trimesters of the pregnancy. Maternal MTHFR *677C>T* and *1298 A>C* genotypes were determined by PCR–RFLP. The associations between deficient dietary intake of vitamin B_{12} (<2.0 μg/d) and folate (<400 μg/d) in the first and third trimesters and maternal polymorphisms of *MTHFR* on anthropometric parameters at birth were estimated using a multivariate linear regression model. During pregnancy, the deficient dietary intake was roughly 60 % for folate and 19 % for vitamin B_{12}. Allelic frequencies of *677T* and *1298C* were 59 and 10 %, respectively. After adjusting for confounders, deficiency in maternal dietary intake of vitamin B_{12} (<2.0 μg/d) was associated with a significant reduction in length ($\beta \sim -2.4$; 95 % CI −4.3; −0.6) and length-for-age at birth ($\beta \sim -1.2$; 95 % CI −2.3; −0.1) among infants whose mothers were carriers of the *677TT* genotype (p for interaction = 0.02). In contrast, no association was observed between deficiency in maternal dietary intake of folate (<400 μg/d) and any anthropometric parameter of newborns. These results suggest that supplementation with vitamin B_{12} during pregnancy could have a favorable impact on intrauterine fetal development mainly in populations that are genetically susceptible.

Keywords Folate and vitamin B_{12} · Gene mutations · MTHFR polymorphism · Newborn's anthropometric parameters · Z-scores

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Introduction

The metabolism of folic acid and vitamin B_{12} is interrelated and both vitamins, along with the flavoprotein methylenetetrahydrofolate reductase (MTHFR), participate in the synthesis of methionine from homocysteine. MTHFR catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the methyl donor for methionine synthesis from homocysteine (Hcy). This reaction is catalyzed by methionine synthase and uses vitamin B_{12} as a cofactor. Methionine is the precursor of S-adenosylmethionine, the methyl group donor necessary for DNA synthesis, cell division, tissue growth, among other reactions. Methionine is also essential for DNA methylation as it plays an important role during critical periods of growth and development (Rush et al. 2014). Two common polymorphisms in the gene coding for the MTHFR (677 C>T and 1298 A>C) are associated with decreased activity of the enzyme (van der Put et al. 1998). In combination with these polymorphisms, a depleted status of folate and vitamin B_{12} inhibits the regeneration of methionine which results in higher concentrations of Hcy and its metabolites (Cortese and Motti 2001).

The association between maternal status of folate and vitamin B_{12} during pregnancy with birth weight has been evaluated separately using biological samples and dietary intake, which has yielded contradictory results. In relation to folate, some studies suggest a negative association between high maternal folate status and risk of low birth weight or small-for-gestational-age babies (van Uitert and Steegers-Theunissen 2013). Meanwhile, vitamin B_{12} deficiency has been associated with reduced birth weight (Wadhwani et al. 2013), but no association with small-for-gestational-age babies has been found (Abraham et al. 2013).

Studies evaluating the MTHFR polymorphisms on infants' anthropometric parameters suggest that *MTHFR 677 C>T* polymorphism is associated with low birth weight. However, only two studies addressed the joint role of maternal status of folate and vitamin B_{12} and *MTHFR 677 C>T* polymorphisms on birth weight. Kordas et al. (2009) reported a significant increase of five grams in birth weight for each μg/d increase in maternal folate intake among women carriers of allele *677 T*; they did not evaluate vitamin B_{12} intake. Meanwhile, Sukla et al. (2013) found that the maternal *MTHFR 677T* allele and vitamin B_{12} and folate deficiencies were independently associated with a twofold increased risk of low birth weight in Indian children. Women with *MTHFR 1298 A>C* polymorphism have an increased risk of placental vasculopathies (Klai et al. 2011) and idiopathic recurrent early pregnancy loss (Cao et al. 2014); however, available information on this polymorphism and infant anthropometric parameters is scarce.

Among the Mexican population, the *677T* allele frequency is ~50 %, which is one of the highest in the world (Guéant-Rodriguez et al. 2006). According to estimates by the National Nutrition Survey, in Mexico, 34.7 % of women aged 12–49 years have an inadequate consumption of folate (Barquera et al. 2003). Although there is no information about the deficiency of vitamin B_{12} intake at the population level, in previous reports with non-pregnant women of reproductive age in the same population, we found a dietary vitamin B_{12} deficiency of 15.4 % (Torres-Sánchez et al. 2006) and 21.3 % of pregnant women during the first trimester of pregnancy (Del Río-García et al. 2009).

The objective of this study was to evaluate the effect of maternal dietary intake of vitamin B_{12} and folate during pregnancy and their interactions with maternal *MTHFR* polymorphism on length and anthropometric z-scores of the newborns.

Materials and methods

Study population

A prospective cohort study was conducted from January 2001 to June 2005 with 996 reproductive age women living in the State of Morelos, Mexico. The original objective of the cohort was to evaluate the association between prenatal organochlorine exposure and infant neurodevelopment; detailed information on the cohort selection methods has been published elsewhere (Torres-Sánchez et al. 2007). The women were identified during prenuptial talks, which are required by law for civil marriages. The eligibility criteria included: reproductive age, intention of living in one of the selected counties during the upcoming 2 years, not breastfeeding, not using an anticonvulsive drug, no history of chronic disease and not using a permanent contraceptive method. A structured pre-pregnancy interview related to sociodemographic characteristics, diet and reproductive history was administered to each participant upon entering the study. After that, follow-up was conducted until they became pregnant and the evolution of the pregnancy was monitored thereafter.

After 8.5 years of follow-up, a total of 442 births occurred, which were followed from birth to 60 months of age. Children eligible for this study ($n = 231$) were those from mothers older than 15 years of age with no delivery complications and who had complete dietary information and *MTHFR* genotyping.

Ethical aspect

This study was approved by the National Institute of Public Health's Ethics Committee and informed consent was obtained from study participants at baseline.

Prenatal evaluation

The women were visited each trimester during pregnancy, and maternal information was collected related to weight, height, diet and tobacco use. Dietary information was obtained using a previously validated semi-quantitative food frequency questionnaire during the first and third trimesters of pregnancy (Galvan-Portillo et al. 2007). The frequency of consumption of 95 items, with predetermined portions, was classified according to ten response categories from 'never' up to 'six times per day.' Nutrient intake of folate, vitamin B_{12}, calcium, iron and zinc was estimated using a computer program developed by the University of Texas [Food Intake Analysis System (FIAS) 3.0; Houston, USA]. The main dietary sources of folate included in the questionnaire were as follows: liver, beans, lima beans, lentils, sweet peas, tomatoes, spinach, lettuce, potato, cauliflower, broccoli, squash flowers, corn, chili, zucchini, squash, beets, carrots, avocado, orange, papaya, mango, strawberries, banana, tangerine, melon, watermelon, blackberries, pears, pineapple, apple, peaches, grapes, tuna, pork, lamb, beef, eggs and dairy. Dietary sources of vitamin B_{12} included: liver, sardines, tuna, pork, lamb, beef,

dairy, eggs, fish, chocolate, beer and white and red wines. This methodology is described in two previous studies where we used this program (Torres-Sánchez et al. 2006; Del Río-García et al. 2009).

MTHFR genotyping

One blood sample was drawn at some point during pregnancy. These whole blood samples were immediately put on ice and centrifuged within 12 h at 2,000 rpm for 10 min. Genomic DNA was isolated from buffy coat with Qiagen Miniprep Kit following the manufacturer's instructions. Genotyping for *MTHFR 677C>T* and *MTHFR 1298A>C* polymorphism has been previously published (Chen et al. 1996; Weisberg et al. 1998). Routine positive (with known allele status) and negative (no DNA) PCR controls were included in every batch of PCR samples. Blind tests were also carried out on 10 % of the samples in each dataset as an additional quality control measure.

Due to financial restriction, maternal blood lead levels were determined in one subsample ($n = 117$ and $n = 134$, first and third trimester, respectively). Using a voltammetric anode separation method, a duplicate analysis of blood lead levels was performed in ESA laboratories (Environmental Science Associates Laboratories, Inc., Chelmsford, MA, USA). Samples with mean levels <5 μg/dL were analyzed again using atomic absorption spectrometry (model 3000; Perkin-Elmer Inc., Norwalk, CT, USA). External quality control samples were provided by the Centers for Disease Control and Prevention laboratories (Atlanta, GA, USA) and the Pennsylvania State Blood Lead Proficiency Testing Program (Exton, PA, USA).

Evaluation of children

At 1 month of age, questions were collected from mothers regarding gestational age, birth weight, birth length and maternal and/or child complications (e.g., perinatal hypoxia). At the time of the interview, most of the mothers did not have hospital discharge records, so self-reported responses were used. In cases in which the hospital discharge records were available both sources of information were compared.

Statistical analysis

To classify inadequate consumption of essential nutrients by pregnant Mexican women, the following cutoffs were used: <400 μg/day for folate, <2.0 μg/day for vitamin B_{12}, <30 mg/day for iron, <15 mg/day for zinc and <1,200 mg/day for calcium (Ávila Curiel 2002).

Z-scores were calculated for all anthropometric measurements at birth (weight-for-age, length-for-age, weight-for-length and BMI-for-age) using WHO Anthro, free software based on the World Health Organization (WHO) standards for children from birth to 5 years old (WHO 2006).

To evaluate the association between maternal dietary intake of folate and vitamin B_{12} with the anthropometric measurements taken at birth, separate linear regression models were generated for each trimester of pregnancy and each dependent variable (birth weight, length-at-birth, weight-for-age, length-for-age, weight-for-length and BMI-for-age).

The following known risk factors for fetal development were selected as potential confounders: maternal age (years), height (cm), education (years), paid occupation (yes/no), parity (none/1–2) and body mass index during the first trimester of pregnancy (kg/m^2) and usage of vitamin supplements during pregnancy. Dietary intake of calcium (mg/day), iron (mg/day) and zinc (mg/day), and caloric intake were also assessed during the first and third trimesters of pregnancy. In addition, information was obtained about smoking status (non-smoker/not a passive smoker, passive smoker before and during first trimester of pregnancy, and smoker before and during first trimester of pregnancy), gestational age and sex of child (female/male). In the final adjusted models, only those variables that modified the coefficients by more than 10 % remained in the model. In a subsample, the final models were ran and also adjusted by maternal lead levels.

To evaluate the interactions between maternal folate and vitamin B_{12} intake with *MTHFR 677C>T and MTHFR 1298 A>C polymorphism* on anthropometric parameters at birth, interaction terms were generated with these variables.

To reduce the possibility of a type I error, bootstrap resampling was performed and the corrected beta estimates were reported. The diagnosis of the models consisted of the estimation of residuals by subtracting the recorded values for each anthropometric parameter from the model's linear predictions for those values. Residual normality was evaluated with Shapiro-Wilks and Shapiro-Francia tests, histograms and normal quantile graphs. We also graphed the model predictions versus standardized residuals to evaluate the residual homoscedasticity. In addition, we checked the heteroskedasticity using the Cook-Weisberg test. All analyses were performed using STATA 13 (StataCorp, College Station, TX, USA).

Results

Selected characteristics of study population are shown in Table 1. Regarding maternal intake of nutrients during pregnancy (Table 2), more than 50 % of the participants

Table 1 Selected maternal and child characteristics of the study population

	($n = 231$)	Percentiles	
		10th	90th
Maternal characteristic			
Age (years)			
Mean ± SD	21.62 ± 3.82	17	27
Education (years)			
Mean ± SD	10.70 ± 3.09	6	15
Parity > 1(%)	16.45		
Paid occupation (%)	44.59		
BMI at first trimester			
Mean ± SD	23.14 ± 3.82	18.73	28.81
Tobacco smoking (%)			
Non-smoker	175 (75.76)		
Passive smoker	38 (16.45)		
Smoker	18 (7.79)		
Supplement intake (%)			
Yes	142 (61.47)		
No	89 (38.53)		
Hypertension (%)			
Yes	20 (8.66)		
No	211 (91.34)		
Child characteristics			
Male (%)	133 (57.58)		
Gestational age (weeks)	39.24 ± 1.58	37.30	41.08
<37 weeks (%)	13 (5.63)		
Birth weight (g)	3.21 ± 0.46	2.65	3.80
Birth length (cm)	50.36 ± 2.38	47.0	53.0
Z-score at birth			
Weight-for-age	−0.22 ± 0.95	−1.35	0.98
Length-for-age	0.40 ± 1.22	−1.15	1.65
BMI-for-age	−0.65 ± 1.41	−2.27	1.02
Weight-for-length	−0.87 ± 1.63	−2.85	1.23

Table 2 Maternal dietary intake of folate and vitamin B_{12} and MTHFR genotypes

Maternal characteristic	First trimester ($n = 231$)	Third trimester ($n = 231$)
Nutrients dietary intake (%)		
Energy (Kcal/d)		
Mean ± SD	2,822.84 ± 902.75	2,809.76 ± 892.65
Folate (<400 μg/d)	144 (62.34 %)	147 (63.64 %)
B_{12} Vitamin (<2.0 μg/d)	43 (18.61 %)	44 (19.05 %)
Calcium (<1,200 mg/d)	143 (61.90 %)	130 (56.28 %)
Iron (<30 mg/d)	208 (90.04 %)	212 (91.77 %)
Zinc (<15 mg/d)	220 (95.24 %)	228 (98.70 %)
MTHFR genotypes (%)		
MTHFR 677 C>T[a]		
CC	36 (15.58)	–
CT	115 (49.78)	–
TT	80 (34.63)	–
MTHFR 677 C>T		
C	187 (40.48)	–
T	275 (59.52)	–
MTHFR 1298A>C[b]		
AA	187 (81.66)	–
AC	38 (16.59)	–
CC	4 (1.75)	–
MTHFR 1298 A>C		
A	412 (89.96)	
C	46 (10.04)	

[a] Hardy–Weinberg equilibrium test = 0.61

[b] Hardy–Weinberg equilibrium test = 0.25

were below the recommended intake values for all nutrients except vitamin B_{12} (19.05 %); no significant differences were observed between the first and third trimesters of pregnancy. In relation to *MTHFR 677C>T* polymorphism, 49.78 % of women were carriers of the heterozygote genotype (*677CT*) and 34.63 % were homozygotes (*677TT*); the allelic frequency of the *677T* variant was 59.52 %. For the MTHFR 1298A>C polymorphism, 81.66 % of women were carriers of the *1298AA* genotype and the allelic frequency of the *1298C* was 10.04 %. Combined heterozygosity (*677C>T + 1298A>C*) was observed in 21.93 % of women. Both genotypes were in agreement with the Hardy–Weinberg equilibrium ($p > 0.05$).

Low maternal folate intake (<400 μg of folate per day) during the third trimester of pregnancy was associated with lower birth weight ($\Delta_{mean} = -120.15$ g; SE = 0.06), lower weight-for-age and BMI-for-age. Deficient intake of vitamin B_{12} during the first trimester of pregnancy was significantly associated with shorter length (49.62 vs. 50.53 cm) and lower length-for-age (Table 3).

Regarding the *MTHFR* genotype, the z-score for length-for-age at birth was significantly lower for children whose mothers were carriers of the *677TT* genotype than for those whose mothers were carriers of the *677CC+CT* genotype (0.19 vs. 0.51) (Table 4). No significant effect was observed with *MTHFR 1298A>C* polymorphism.

After adjusting separately for potential confounding variables in each model and correcting by bootstrap re-sampling, children of mothers with a deficient dietary intake of vitamin B_{12} during the first trimester of pregnancy had a significantly lower length (β = −0.88, 95 % CI −1.71; −0.04) and significantly lower z-scores for length-for-age (β = −0.45, 95 % CI −0.89; −0.02) than children of mothers with adequate dietary intake (Table 5). When stratified by maternal *MTHFR* genotype, these

Table 3 Infant anthropometric parameters at birth (mean ± SD) according to maternal dietary intake of folate and vitamin B_{12}

Infant anthropometrics parameters at birth	Folate intake (μg/d)				Vitamin B_{12} intake (μg/d)			
	First trimester		Third trimester		First trimester		Third trimester	
	< 400 $n = 144$	≥ 400 $n = 87$	< 400 $n = 147$	≥ 400 $n = 84$	<2.0 $n = 43$	≥2.0 $n = 188$	<2.0 $n = 44$	≥2.0 $n = 187$
Weight (kg)	3.22 ± 0.47	3.20 ± 0.44	3.17* ± 0.46	3.29 ± 0.44	3.12 ± 0.41	3.23 ± 0.47	3.11 ± 0.42	3.24 ± 0.47
Length (cm)	50.27 ± 2.36	50.50 ± 2.41	50.32 ± 2.35	50.43 ± 2.45	49.62 ± 2.58*	50.53 ± 2.31	49.99 ± 2.46	50.45 ± 2.36
BMI	12.80 ± 1.80	12.55 ± 1.51	12.57 ± 1.77	12.94 ± 1.54	12.77 ± 2.12	12.69 ± 1.59	12.52 ± 1.92	12.75 ± 1.64
Z-Score at birth								
Weight-for-age	0.21 ± 0.96	0.26 ± 0.94	0.31 ± 0.96*	0.06 ± 0.93	0.38 ± 0.88	0.18 ± 0.97	0.40 ± 0.93	0.18 ± 0.96
Length-for-age	0.35 ± 1.19	0.48 ± 1.27	0.37 ± 1.19	0.45 ± 1.28	0.07 ± 1.36*	0.48 ± 1.18	0.27 ± 1.30	0.43 ± 1.21
BMI-for-age	−0.58 ± 1.46	−0.77 ± 1.31	−0.77 ± 1.48*	−0.44 ± 1.24	−0.64 ± 1.64	−0.65 ± 1.36	−0.83 ± 1.50	−0.61 ± 1.39
Weight-for-length	−0.79 ± 1.66	−0.99 ± 1.58	−0.97 ± 1.71	−0.69 ± 1.47	−0.91 ± 1.70	−0.86 ± 1.62	−1.09 ± 1.55	−0.82 ± 1.65

* p for t test < 0.05

associations remained significant only among children whose mothers were carriers of MTHFR *677TT* genotype, but not among those whose mothers were carriers of MTHFR *677 CC+CT* genotype (p for interactions < 0.05). No association was observed for children of women with deficient dietary intake of folate (<400 μg/d); likewise no interaction with *MTHFR 1298 A>C* polymorphism was observed.

For a subsample, the maternal blood lead geometric means were similar during the first and third trimester of pregnancy (5.23 ± 1.89 vs. 5.55 ± 1.90 μg/dL) and did not confound the associations between length (first trimester: $\beta_{\text{not adjusted by lead}} = -1.78$ vs. $\beta_{\text{adjusted by lead}} = -1.74$; third trimester $\beta_{\text{not adjusted by lead}} = -1.01$ vs. $\beta_{\text{adjusted by lead}} = -1.01$) and length-for-age (first trimester: $\beta_{\text{not adjusted by lead}} = -0.73$ vs. $\beta_{\text{adjusted by lead}} = -0.71$; third trimester: $\beta_{\text{not adjusted by lead}} = -0.39$ vs. $\beta_{\text{adjusted by lead}} = -0.38$) observed among women carriers of *677 TT* genotypes.

Discussion

This study is one of few to simultaneously evaluate the effect of dietary intake of folate and vitamin B_{12} and *MTHFR 677 C>T* polymorphism on newborn's anthropometric parameters. The results show that a dietary deficiency of vitamin B_{12} during pregnancy is associated with significantly smaller size-at-birth, mainly for children whose mothers are carriers of the *677TT* genotype. Dietary consumption of folate during pregnancy and *MTHFR 1298 A>C* polymorphism were not associated with any of the anthropometric parameters studied.

Due to methodological differences, the comparison of our results among other studies was difficult. However, regarding to those that evaluated dietary intake of folate, our results are consistent with those reported by Kordas et al. (2009), whose study of a pregnant cohort in Mexico City did not observe significant differences in birth weight and size-at-birth for children whose mothers consumed <400 or ≥400 μg/d of folate. Nevertheless, they reported a significant increase of 5 g in birth weight for every 100 μg/d increase in dietary intake of folate. In a cohort study conducted in Norway, Nilsen et al. (2010) also did not find a significant association between dietary intake of folate, consumption of folic acid supplements and plasma concentration of folate with gestational weight-for-age. In contrast, Pastor Valero et al. (2011), who defined small-for-gestational-age for weight (SGA-W) and for height (SGA-H) as below the 10th percentile, found that women in the highest quintile of dietary intake of folate had a significantly lower risk of an SGA-W baby; however, they did not observe any association with SGA-H.

Table 4 Newborn's anthropometric parameters (Mean ± SD) according to maternal MTHFR genotypes

Newborn's anthropometrics parameters	*MTHFR 677 C>T*		*MTHFR 1298 A>C*	
	TT ($n = 80$)	*CC+CT* ($n = 151$)	*AA* ($n = 187$)	*AC+CC* ($n = 42$)
Weight (kg)	3.17 ± 0.47	3.24 ± 0.45	3.19 ± 0.46	3.29 ± 0.47
Length (cm)	50.02 ± 2.58	50.54 ± 2.25	50.30 ± 2.51	50.61 ± 1.63
BMI	12.81 ± 1.85	12.65 ± 1.62	12.70 ± 1.75	12.73 ± 1.49
Z-score at birth				
Weight-for-age	−0.29 ± 0.95	−0.18 ± 0.96	−0.25 ± 0.97	−0.10 ± 0.88
Length-for-age	0.19 ± 1.30*	0.51 ± 1.17	0.36 ± 1.29	0.56 ± 0.86
BMI-for-age	−0.57 ± 1.50	−0.69 ± 1.36	−0.66 ± 1.45	−0.60 ± 1.25
Weight-for-length	−0.74 ± 1.69	−0.94 ± 1.60	−0.88 ± 1.68	−0.80 ± 1.42

* p for t test < 0.05

Few studies examine the relationship between dietary intake of vitamin B_{12} during pregnancy and anthropometric parameters at birth, withal of them focusing solely on birth weight. Takimoto et al. (2011) reported a significant increase in birth weight ($\beta = 0.08$; 95 % CI 0.01, 0.15) associated with dietary intake of vitamin B_{12} during the third trimester of pregnancy, while Sukla et al. (2013) observed that women with deficient plasma vitamin B_{12} were two times more likely of having a low weight infant than those without this deficiency. Other authors did not find an association between vitamin B_{12} and birth weight (Relton et al. 2005, Bergen et al. 2012).

Vitamin B_{12} along with 5-methyltetrahydrofolate and methionine synthase enzyme participates in the transformation of Hcy to methionine. Methionine metabolizes to S-adenosylmethionine, which acts as a methyl donor in many reactions, including the methylation of DNA, histones and other proteins (Rush et al. 2014). Pregnant women carriers of *677C>T* polymorphism in MTHFR gene have an enzymatic variant with less activity and, therefore, less 5-methyltetrahydrofolate availability. In these women, a deficient dietary intake of vitamin B_{12} can produce an increase of maternal serum Hcy levels, which during pregnancy have been related with alterations in the vascular endothelium at the placental level, leading to adverse reproductive effects such as preeclampsia, miscarriage and restricted fetal growth (Bergen et al. 2012). Another potential mechanism is that vitamin B_{12} deficiency reduces the methionine metabolism and can affect the patterns of epigenetic modifications such as DNA methylation that alter the gene expression and thus affect the fetal development (Rush et al. 2014).

We do not rule out an association between folate and newborn's anthropometric parameters. The high frequency observed in dietary deficiency of folate and the possibility of a non-differential measurement error in the calculation of micronutrient intake could result in underestimating the associations evaluated, particularly for birth weight. This type of error is more common when a food frequency questionnaire is used to evaluate maternal nutritional status instead of biomarkers. In addition, the lack of detailed information on the amount of folic acid in prenatal vitamins made difficult the assessment of maternal folate status.

Information about length and weight at birth were mainly obtained through questionnaire; however, a previous study performed in Mexican population showed a high correlation (rho = 0.93) between this source of information and that contained into hospital records (Moreno-Banda et al. 2009). Additionally, since mothers and the persons conducting the measurements were not aware of the study's hypothesis, dietary deficiencies of vitamin B_{12} or maternal genotype, it was virtually impossible for either of the two anthropometric measurements to have been differentially measured or reported as a function of both characteristics.

With respect to the evaluation and control of confounders, the possible independent factors that affect fetal growth, according to the literature, were considered by the analysis as potential confounders. Since prenatal exposure to lead is associated with a reduction in size-at-birth, the possibility of this being a confounder was evaluated only in a sub-sample of women and the coefficients associated with size and size-for-age showed practically no change. Regarding to MTHFR 1298 A>C role on fetal growth, it is not possible to reject any association or interaction with maternal folate and vitamin B_{12} status due to the small allelic polymorphic frequency.

The evaluation of maternal diet during the first and third trimesters of pregnancy is one of the strengths of the study, since this made it possible to identify changes in diet during pregnancy. These two periods of pregnancy correspond to the organogenesis and maximum fetal growth stages, which are key to the size and weight observed at birth.

Conclusion

In Mexico, our results are relevant since the frequency of *677C>T* polymorphism is one of the highest worldwide

Table 5 Association between maternal intake of folate and vitamin B_{12} and newborn's anthropometric parameters according MTHFR 677 C>T genotypes

Newborn's anthropometric Parameters															
MTHFR 677 C > T	Dietary intake	Weight[a] (kg)		Length[a] (cm)		BMI[a]		Z-Score at birth[b]							
								Weight-for-age		Length-for-age		BMI-for-age		Weight-for-length	
		β	95 % CI	β	95 % CI	β	95 % CI	β	95 % CI	β	95 % CI	β	95 % CI	β	95 % CI
All[c]	Folate (<400 μg/d)														
	First trimester ($n = 144$)	0.07	−0.05; 0.20	0.15	−0.53; 0.84	0.26	−0.23; 0.76	0.17	−0.11; 0.44	0.07	−0.29; 0.43	0.22	−0.19; 0.62	0.17	−0.30; 0.64
	Third trimester ($n = 147$)	−0.09	−0.22; 0.03	−0.02	−0.68; 0.64	−0.27	−0.75; 0.21	−0.18	−0.44; 0.08	−0.05	−0.40; 0.30	−0.22	−0.62; 0.17	−0.17	−0.63; 0.28
	Vitamin B_{12} (<2.0 μg/d)														
	First trimester ($n = 43$)	−0.09	−0.24; 0.07	−0.88	−1.71; −0.04*	0.12	−0.49; 0.74	−0.24	−0.57; 0.10	−0.45	−0.89; −0.02*	−0.007	−0.51; 0.49	−0.06	−0.65; 0.52
	Third trimester ($n = 44$)	−1.0	−0.27; 0.07	−0.63	−0.15; 0.28	−0.01	−0.68; 0.65	−0.27	−0.63; 0.08	−0.38	−0.85; 0.08	−0.12	−0.65; 0.41	−0.11	−0.73; 0.51
TT	Folate (<400 μg/d)														
	First trimester ($n = 55$)	0.11	−0.10; 0.32	−0.04	−1.30; 1.23	0.44	−0.38; 1.27	0.29	−0.16; 0.74	0.14	−0.45; 0.73	0.37	−0.30; 1.04	0.31	−0.48; 1.10
	Third trimester ($n = 52$)	−0.18	−0.39; 0.02	−0.40	−1.62; 0.82	−0.34	−1.14; 0.46	−0.30	−0.74; 0.14	−0.23	−0.81; 0.35	−0.22	−0.87; 0.43	−0.23	−0.99; 0.53
	Vitamin B_{12} (<2.0 μg/d)														
	First trimester ($n = 13$)	−0.13	−0.40; 0.14	−2.21	−4.21; −0.22**	0.56	−0.49; 1.61	−0.38	−0.95; 0.19	−1.12	−2.04; −0.21**	0.32	−0.53; 1.18	0.27	−0.75; 1.29
	Third trimester ($n = 12$)	−0.06	−0.37; 0.24	−2.47	−4.30; −0.64**	1.08	−0.09; 2.24	−0.10	−0.74; 0.53	−1.20	−2.30; −0.11**	0.83	−0.55; 2.21	0.76	−0.27; 1.78
CC+CT	Folate (<400 μg/d)														
	First trimester ($n = 89$)	0.08	−0.08; 0.24	−0.19	−0.91; 0.53	0.33	−0.27; 0.93	0.17	−0.16; 0.51	−0.003	−0.43; 0.43	0.28	−0.21; 0.78	0.24	−0.35; 0.85
	Third trimester ($n = 95$)	−0.02	−0.17; 0.13	0.02	−0.71; 0.75	−0.14	−0.70; 0.45	−0.07	−0.39; 0.25	0.02	−0.39; 0.43	−0.14	−0.61; 0.34	−0.05	−0.62; 0.53
	Vitamin B_{12} (<2.0 μg/d)b12														
	First trimester ($n = 32$)	−0.07	−0.27; 0.12	−0.37	−1.34; 0.60	0.04	−0.69; 0.77	−0.13	−0.54; 0.27	−0.22	−0.74; 0.30	−0.03	−0.64; 0.57	−0.09	−0.82; 0.64
	Third trimester ($n = 32$)	−0.14	−0.34; 0.06	−0.14	−1.18; 0.89	−0.49	−1.26; 0.27	−0.35	−0.77; 0.07	−0.14	−0.69; 0.40	−0.45	−1.07; 0.18	−0.34	−1.09; 0.41

[a] Adjusted by: maternal age, schooling, parity, BMI during first trimester, energy, calcium, iron and zinc dietary intake, gestational age (weeks), sex of child and vitamin supplementation during pregnancy (yes/no)

[b] Adjusted by: maternal age, schooling, parity, BMI during first trimester, energy, calcium, iron and zinc dietary intake and vitamin supplementation during pregnancy (yes/no)

[c] Adjusted by MTHFR 677 C>T

* $p < 0.05$

** p for interaction < 0.05

(~50 %) and can theoretically increase the susceptibility of the population to the adverse reproductive effects caused by nutritional deficiencies, such as those related to the metabolism of methyl groups. In the offspring, low maternal vitamin B_{12} status during pregnancy is associated with increased risk of neural tube defect, low lean mass and excess adiposity, increased insulin resistance, impaired neurodevelopment and altered risk of cancer (Rush et al. 2014). In addition, children born with alteration of growth fetal development had a greater risk during adulthood of metabolic disorders and chronic illnesses (Darendeliler et al. 2008).

The current standard for prenatal care promotes preventive use of folic acid supplementation, but not the vitamin B_{12}. Nevertheless, given the evidence of the role this vitamin has in fetal growth and the effects mentioned, the replication of this type of studied is recommended to compensate for the limitations of the present study in order to provide a basis for decision-making related to vitamin B_{12} supplementation during pregnancy.

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Conflict of interest Luisa Torres-Sánchez, Lizbeth López-Carrillo, Julia Blanco-Muñoz and Jia Chen declare that they have no conflict of interest.

Ethical standard All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (National Institute of Public Health of Mexico) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study.

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