

Periconception folic acid supplementation, fetal growth and the risks of low birth weight and preterm birth: the Generation R Study

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Countries worldwide, including the Netherlands, recommend that women planning pregnancy use a folic acid supplement during the periconception period. Some countries even fortify staple foods with folic acid. These recommendations mainly focus on the prevention of neural tube defects, despite increasing evidence that folic acid may also influence birth weight. We examined whether periconception folic acid supplementation affects fetal growth and the risks of low birth weight, small for gestational age (SGA) and preterm birth, in the Generation R Study in Rotterdam, the Netherlands. Main outcome measures were fetal growth measured in mid- and late pregnancy by ultrasound, birth weight, SGA and preterm birth in relation to periconception folic supplementation (0.4–0.5 mg). Data on 6353 pregnancies were available. Periconception folic acid supplementation was positively associated with fetal growth. Preconception folic acid supplementation was associated with 68 g higher birth weight (95 % CI 37.2, 99.0) and 13 g higher placental weight (95 % CI 1.1, 25.5), compared to no folic acid supplementation. In these analyses parity significantly modified the effect estimates. Start of folic acid supplementation after pregnancy confirmation was associated with a reduced risk of low birth weight (OR 0.61, 95 % CI 0.40, 0.94). Similarly, reduced risks for low birth weight and SGA were observed for women who started supplementation preconceptionally, compared to those who did not use folic acid (OR 0.43, 95 % CI 0.28, 0.69 and OR 0.40, 95 % CI 0.22, 0.72). In conclusion, periconception folic acid supplementation is associated with increased fetal growth resulting in higher placental and birth weight, and decreased risks of low birth weight and SGA.

Folate: Birth weight: Epigenetics: Effect modification

Low birth weight, as a proxy for fetal growth, is associated with various chronic diseases later in life^(1,2). Fetal growth depends on multiple genetic factors and environmental exposures, derived from the mother. In this respect, maternal nutrition during pregnancy has been shown to play a critical role^(1,3,4).

Because developing organ systems directly respond with permanent adaptations to the availability of nutrients during critical periods of rapid development, timing of adequate maternal nutrition is important to determine the effects both in the fetus and child^(2,4–6). Moreover, first evidence has suggested that fetal growth is vulnerable to maternal nutrition, especially during the preconception period and first weeks of gestation, since it has the potential to affect epigenetic mechanisms in the placenta and fetus^(3,4,7–9). Folate is for this reason of great interest. Together with vitamin B₁₂ it plays a critical role in homocysteine metabolism. The folate-dependent homocysteine pathway is important for protein, lipid

and DNA synthesis. In addition, folate provides methyl groups for the synthesis of methionine and its derivative S-adenosyl-methionine. The latter is the most important methyl donor in the human body for DNA methylation, and represents one of the best known epigenetic mechanisms^(10,11).

During pregnancy folate demand increases because of placental and fetal growth and development⁽¹⁰⁾. For this reason pregnant women with a folate deficiency are at an increased risk for various reproductive failures, including neural tube defects and congenital malformations^(12–16). As a consequence, most European countries, including the Netherlands, recommend that women planning pregnancy use a folic acid supplement during the periconception period in addition to a healthy diet^(17,18). Several studies have shown positive associations between maternal folic acid intake and fetal growth^(19–27). However, the majority of these studies were focused on the effects of folic acid on fetal growth in mid- and late pregnancy. Only few studies assessed the associations

Abbreviation: SGA, small for gestational age.

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in early pregnancy and failed to be consistent^(23,28). Thus, despite the fact that early pregnancy is the most important period for placental development, embryogenesis and fetal programming, relatively little is known about the implications of folic acid supplementation during this particular pregnancy period on fetal growth^(7,8,29,30).

The aim of the present study was to investigate the effects of low-dose periconception folic acid supplementation on fetal and placental growth in low-risk singleton pregnancies. Also, we studied the associations between periconception folic acid intake and low birth weight, small for gestational age (SGA) and preterm birth.

Methods

Design

The study was embedded in the Generation R Study, Rotterdam, the Netherlands, a population-based prospective cohort study from early pregnancy onwards. The Generation R Study was designed to identify early environmental and genetic determinants of growth, development and health from fetal life until young adulthood. The Generation R Study is conducted in Rotterdam, the second largest city in the Netherlands, and eligible women were those who were resident in the study area and delivered between April 2002 and January 2006. The study aimed to enrol women in early pregnancy (gestational age <18 weeks), but enrolment was possible until the birth of the child. All midwifery practices and three hospitals located in the study area participated during the prenatal phase. The overall response rate was about 61%, and is based on the number of children born to eligible mothers during the inclusion period. The Medical Ethics Committee of the Erasmus Medical Centre has approved the study, and all participants gave their written informed consent^(31,32).

Folic acid use

Pregnant women were asked by questionnaire at enrolment (median 15.4 weeks of gestation, 95% range 10.2–24.8) whether they had used a folic acid supplement periconceptionally (folic acid dosage of 0.4–0.5 mg/d, according to the advice of the Health Council of the Netherlands), and when supplementation was started^(33,34). Self-reported folic acid use was categorized into three groups: (1) preconception start: defined as preconception start of folic acid supplement intake at any moment prior to conception; (2) start before 8 weeks: defined as start of folic acid supplementation from the moment that pregnancy was recognized but before the eighth week of gestation; and (3) no use: defined as no use of folic acid supplementation at all. Because our interest was mainly focused on start, and use, of folic acid supplementation during the period just before and after conception, i.e. the periconception period, we did not include women who only started folic acid supplementation after the eighth week of gestation in our main analysis (*n* 46). However, because this group may present an interesting comparison group, we did include them in a sensitivity analysis. About 15% of the women reported to have used folic acid as part of a multivitamin supplement regimen. The doses of folic acid in these multivitamins were approximately 0.4–0.5 mg/d.

Fetal ultrasonography

Fetal ultrasound examinations were carried out in early pregnancy (median 13.5 week of gestation, 95% range 10.6–17.5), mid-pregnancy (median 20.6 weeks of gestation, 95% range 18.6–23.4) and late pregnancy (median 30.4 weeks of gestation, 95% range 28.4–33.0). The ultrasound examinations were used for establishing both gestational age and fetal growth characteristics⁽³⁵⁾. Fetal biometry, including head circumference, biparietal diameter, abdominal circumference and femur length, was measured transabdominally during each ultrasound examination. Crown–rump length was measured in early pregnancy. Dating of pregnancy was performed using the first ultrasound measurement of either crown–rump length (gestational age until 12 weeks and 5 d of gestation, and crown–rump length measurement smaller than 65 mm), or biparietal diameter (gestational age from 12 weeks and 5 d of gestation onwards, and biparietal diameter larger than 23 mm)⁽³⁵⁾. Estimated fetal weight was calculated for mid- and late pregnancy using the formula of Hadlock *et al.*⁽³⁶⁾. Longitudinal growth curves and gestational age-adjusted standard deviation scores were constructed for all fetal growth measurements. These gestational age-adjusted standard deviation scores were based on reference growth curves from the whole study population, and represent the equivalent of *z*-scores⁽³⁵⁾.

Birth outcomes

Medical records completed by community midwives and obstetricians were used to obtain information about gestational age at birth (weeks), birth weight (g), gender and placental weight (g), and to calculate placental index (placental weight/birth weight)^(37,38). Prematurity was defined as the birth of an infant before 37.0 weeks of gestation, and low birth weight was defined as a birth weight <2500 g. Being SGA was defined as a standard deviation score ≤ -2.3 (<5th percentile) and based on standard deviation curves derived from this cohort⁽³⁵⁾.

Covariates

Information about maternal age, educational level, ethnicity, smoking and parity was obtained from the questionnaire at enrolment in the study. Educational level was assessed by the highest completed educational level of the mother and classified into two categories: (1) primary school; and (2) secondary school, university or college^(32,39). Ethnic background was defined from information from the country of birth of the woman herself and her parents and categorized as follows: (1) western, including Dutch, European, North American, and Oceanian; (2) Moroccan; (3) Turkish; (4) Antillean and Surinamese; and (5) other non-Western, including African, Asian, South American and Central American^(32,40). At the first ultrasound examination, maternal height (m) and weight (kg) were measured and BMI (in kg/m²) was calculated. Information on fertility treatment and pregnancy complications, including pre-eclampsia and gestational diabetes, was obtained from midwives and obstetricians. Women who became pregnant after fertility treatment, including *in vitro* fertilization and intracytoplasmic sperm-injection, were excluded from analyses.

Statistical analyses

First, independent Student's *t* test and Mann–Whitney *U* test were used to test differences in characteristics between women who used periconception folic acid and women who did not use a folic acid supplement. Next, associations of folic acid supplementation with fetal growth characteristics (head circumference, abdominal circumference, femur length, estimated fetal weight) and birth characteristics (birth weight, placental weight) in low-risk singleton pregnancies were assessed using univariate linear regression models (model A). The linear regression analyses that were based on fetal growth characteristics (head circumference, abdominal circumference, femur length and estimated fetal weight) were restricted to those mothers who enrolled and had their pregnancies dated in early pregnancy (78%). The consideration of confounding variables in our analyses was primarily determined *a priori* and based on earlier literature. These included time of enrolment in study, gestational age, maternal age, height, weight, parity, ethnicity, fetal gender, educational level, smoking, alcohol use, primary or secondary antenatal care, gestational diabetes and pre-eclampsia^(41,42). Potential confounders were selected as a result of exploratory analyses and were included in the analyses if the effect estimates of the fetal growth parameters changed more than 10%. By using this approach, type of antenatal care, alcohol consumption, pre-eclampsia and gestational diabetes were not included in the final multivariate regression model (model B). To analyse the associations of folic acid supplementation with the risks of low birth weight, SGA and preterm birth, we used univariate and multivariate logistic regression models, with a similar approach to select confounders. Effect modification was tested by multiplying folic acid supplement use with the covariates educational level, ethnicity, smoking habits, parity and BMI. Subsequently, and under the condition of a *P* value < 0.1, multivariate linear regression analyses were performed in strata of that specific determinant. Last, to test any possible associations of folic acid supplementation started after the eighth week of gestation with fetal growth, we also performed similar analyses with the cohort categorized into four groups, namely (1) preconception start; (2) start before 8 weeks; (3) start from 8 weeks onwards; and (4) no folic acid use. The size of the effect estimates (regression coefficients and OR) are presented with their 95% CI. Statistical analyses were performed using the Statistical Package of Social Sciences version 15.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Between 2002 and 2006, 8880 women enrolled during pregnancy in the Generation R Study⁽³²⁾. Forty-six women (0.5%) started using a folic acid supplement after the eighth week of gestation and information on folic acid supplementation was missing in 25% of the women (*n* 2274). Of the remaining 6560 women, forty-seven *in vitro* fertilization or intracytoplasmic sperm-injection pregnancies, sixty-three twin pregnancies and seventy-seven fetal deaths were identified, and twenty women were lost to follow up. Data on 6353 low-risk singleton pregnancies (71.6%) were available.

Characteristics of the women per folic acid category are presented in Table 1. Of all women 39.2% (*n* 2493) started

folic acid supplementation preconceptionally. Approximately 31% of all women (*n* 1983) started folic acid supplementation after pregnancy recognition, and 29.5% of the women (*n* 1877) did not use folic acid supplementation at all. The age of the cohort ranged from 15.3 to 46.3 years with a median of 29.8 years, and the lowest median age was found in women who did not use folic acid (27.8 years). The percentage of women with a higher educational level was also lowest in this group (26.9%). In the whole cohort the largest ethnic groups were the Dutch and other western (63%). The percentage of women who did not use folic acid was highest among non-western women, including Moroccan, Turkish, Surinam and Antillean women (total of 71.6%).

The associations between folic acid use and fetal growth characteristics in mid- and late pregnancy are presented in Table 2. Periconception folic acid supplementation was associated with trends towards significantly larger head circumference and abdominal circumference, in mid- and late pregnancy, compared to women who did not use folic acid. In addition, similar trends towards larger femur length in mid- and late pregnancy were observed for women who preconceptionally used a folic acid supplement, compared to women who did not use folic acid. However, these effect estimates were not significant.

Figure 1 shows the differences in standard deviation scores (*z*-scores) of fetal weight from mid-pregnancy until birth between the three folic acid categories. No significant differences in standard deviation scores between the folic acid supplementation groups were observed for estimated fetal weight in mid-pregnancy. This effect changed over time with significant higher estimated fetal weight in late pregnancy for women who preconceptionally started folic acid supplementation (SD 0.10, 95% CI 0.02, 0.19), and significant higher birth weight for both women who preconceptionally started folic acid supplementation and women who started after pregnancy recognition, compared to no folic acid supplementation at all (SD 0.16, 95% CI 0.09, 0.23 and SD 0.13, 95% CI 0.06, 0.20, respectively). After adjustment for potential confounders birth weight was 68 g higher in women who preconceptionally started folic acid supplementation (95% CI 37.2, 99.0) and 53 g higher in women who started supplementation after pregnancy recognition (95% CI 23.60, 83.18), compared to birth weight of newborns of women who did not use folic acid supplementation.

Table 3 shows the associations between folic acid use and placental weight. Placental weight was approximately 10 g more in women who preconceptionally started using a folic acid supplement (95% CI 0.32, 20.49) compared to placental weight of women who did not use folic acid supplementation. After adjusting for potential confounders this effect estimate did not change. For placental index, a trend was observed towards a smaller index in both women who used preconception folic acid (β -0.004, 95% CI -0.007, -0.002), as well as in women who started after pregnancy recognition but before the eighth week of gestation (β -0.003, 95% CI -0.006, -0.001), compared to women who did not use folic acid. This trend, however, was not significant anymore after adjusting for potential confounders.

Table 4 shows the associations between folic acid use and birth outcomes. Preconception start of folic acid was associated with a decreased risk of low birth weight (OR 0.47,

Table 1. Characteristics of participants in the study stratified by category of folic acid use (Mean values and standard deviations)

	Folic acid supplementation					
	No use (n 1877)		Start before 8 weeks (n 1983)		Preconception start (n 2493)	
	Mean	SD	Mean	SD	Mean	SD
Median age (years)*	27.8	17.9–39.2	29.7	19.7–39.0	31.5	22.6–39.5
Height (cm)	164.4	7.0	168.1	7.0	169.5	7.0
Weight (kg)	69.6	14.8	69.2	12.9	69.9	12.4
Multigravida (%)		55.8		37.4		39.1
Education (%)						
Primary school		26.9		6.9		3.0
Secondary school, university or college		73.1		93.1		97.0
Ethnicity (%)						
Western		28.4		69.0		83.9
Moroccan		15.5		3.8		2.1
Turkish		17.4		7.2		3.8
Surinam and Antilles		19.6		12.0		5.9
Other non-western		19.1		8.0		4.3
Smoking habits any time in pregnancy (%)		29.4		31.8		16.5
Alcohol consumption any time in pregnancy (%)		32.0		57.9		58.3
Enrolment in study in early pregnancy (%)		67.4		80.0		84.7
Antenatal care during first trimester (%)						
Primary care		90.5		92.5		92.1
Secondary care		7.9		7.5		9.5
Fetal growth ultrasound						
Mid-pregnancy (%)		91.8		95.5		95.7
Head circumference (mm)	179.1	13.5	178.7	13.2	179.0	12.6
Abdominal circumference (mm)	156.6	14.0	156.3	13.8	156.7	12.9
Femur length (mm)	33.6	3.4	33.3	3.2	33.3	3.1
Late pregnancy (%)		94.5		96.5		96.9
Head circumference (mm)	283.2	12.3	284.5	12.2	286.1	11.9
Abdominal circumference (mm)	262.0	16.6	262.8	16.1	265.5	2.9
Femur length (mm)	57.3	3.0	57.4	2.9	57.5	2.9
Birth outcomes						
Median gestational age at birth (weeks)*	39.7	39.7–40.0	39.9	35.9–42.4	39.9	25.7–42.4
Birth weight (g)	3340.2	563.6	3424.7	557.9	3484.1	553.7
Placental weight (g)	628.7	149.5	633.5	146.0	644.4	149.4
Placental index	0.190	0.036	0.187	0.035	0.186	0.035
Male gender (%)		51.8		50.9		48.6

* Median with 95 % range.

95 % CI 0.33, 0.68) as well as a decreased risk of SGA (OR 0.38, 95 % CI 0.23, 0.63), compared to women who did not use folic acid supplementation. After adjustment for potential confounders these effect estimates did not further change. In addition, after adjustment for potential confounders, start of folic acid supplementation after pregnancy recognition was also associated with a decrease of having a child with low birth weight (OR 0.61, 95 % CI 0.40, 0.94), compared to women who did not use a folic acid supplement. Folic acid supplementation (either preconception start, or start after pregnancy confirmation) was not significantly associated with the risk of preterm birth after controlling for confounders.

Parity significantly modified the effect of periconceptional folic acid use on birth weight ($P < 0.001$) (Fig. 2). After adjustment for potential confounders, preconception start of folic acid among multiparous women was associated with about 240 g higher birth weight (95 % CI 195.13, 282.92), compared to nulliparous women who did not use a folic acid supplement. In contrast, preconception start of folic acid among nulliparous women was about 55 g higher (95 % CI 15.44, 94.68), compared to nulliparous women

who did not use a folic acid supplement. We did not observe further significant effect modification on the multiplicative scale by educational level, ethnicity, smoking or BMI.

Last, the analysis performed to test possible associations of folic acid supplementation started after the eighth week of gestation with fetal growth, did not reveal any differences between (1) non-users, preconception users and women who started before eight weeks, and (2) folic acid supplementation from 8 weeks onwards, and similarly, no association of this latter group with fetal growth (likely due to small numbers in this group; n 46).

Discussion

In this prospective population-based cohort study we demonstrate that low-dose periconception folic acid supplementation is associated with increased fetal growth compared to non-users, and that parity significantly modifies the effect. Low-dose periconception folic acid supplementation is also associated with reduced risks of having a child with low birth weight or being SGA at birth. No significant association

Table 2. Associations between periconception folic acid use and fetal growth measured by ultrasound* (Regression coefficients and 95 % confidence intervals)

	Head circumference			Abdominal circumference			Femur length					
	Model A†		Model B‡	Model A†		Model B‡	Model A†		Model B‡			
	RC	95 % CI	RC	95 % CI	RC	95 % CI	RC	95 % CI	RC	95 % CI		
Week of growth measurement and folic acid supplementation												
20 weeks												
No use	Ref.		Ref.		Ref.		Ref.		Ref.		Ref.	
Start before 8 weeks	0.54	0.07, 0.99	0.10	-0.41, 0.60	0.53	-0.67, 1.13	0.05	-0.61, 0.71	-0.04	-0.17, 0.09	0.01	-0.14, 0.16
Preconception start	1.26	0.82, 1.70	0.61	0.09, 1.12	1.31	0.75, 1.88	0.41	-0.27, 1.08	-0.02	-0.14, 0.11	0.04	-0.11, 0.19
30 weeks												
No use	Ref.		Ref.		Ref.		Ref.		Ref.		Ref.	
Start before 8 weeks	1.33	0.63, 2.04	0.63	-0.12, 1.38	0.85	-0.13, 1.84	0.32	-0.75, 1.39	0.07	-0.10, 0.24	0.03	-0.16, 0.21
Preconception start	2.78	2.11, 3.45	1.34	0.57, 2.11	3.32	2.39, 4.25	1.71	0.61, 2.80	0.15	-0.01, 0.31	0.02	-0.18, 0.20

RC, regression coefficient; Ref., reference.

*For details of subjects and procedures, see Methods and Table 1. Results are from linear regression analysis. Values are regression coefficients (in mm) and reflect the difference in growth for each characteristic measured in mid-pregnancy (median 20.5 weeks, 95 % range 18.8–23.1) or late pregnancy (median 30.4 weeks, 95 % range 28.5–32.7), compared to no folic acid use.

†Adjusted for gestational age at time of ultrasound measurement.

‡Adjusted for gestational age at time of ultrasound measurement, maternal age, height, weight, parity, ethnicity, fetal gender, educational level and smoking.

was observed between periconception folic acid supplementation and preterm birth.

Folate is a B vitamin that serves as a substrate in many underlying pathways of cellular processes, including cell multiplication, apoptosis, intracellular signalling and programming, which are all implicated in fetal and placental growth and development^(10,11). Therefore, additional supply of folic acid during pregnancy is likely to influence fetal growth. Furthermore, the placental function is of main importance for the growth and development of the fetus. Thus, when folic acid directly affects placental growth and development, it also indirectly affects fetal growth^(7,21,37,38). Previous studies support the present findings and show positive associations between increased folic acid intake, higher birth weight, and reduced risks of low birth weight and SGA^(19–27). However, the majority of these studies were focused on increased folic acid intake in mid- and late pregnancy. Since fetal growth is greatest in the second half of pregnancy, and a larger supply of folic acid during this particular period may directly serve as a substrate for increased cellular synthesis, their results were not really unanticipated⁽⁴³⁾. Only a few studies assessed the associations between folate in early pregnancy and fetal growth^(23,28). Rolschau *et al.*⁽²³⁾ found an increased birth weight in infants of mothers who preconceptionally started folic acid supplementation. Interestingly, they reported this effect only to be present in week 43 of gestation. They also reported reduced prevalences of low birth weight and SGA in newborns of women who preconceptionally started supplementation and, in contrast with the present study, a reduced risk for preterm birth. However, like most other studies that investigated associations between folic acid supplementation and fetal growth, they used very high doses of folic acid (up to 2.5 mg), compared with the low doses of 0.4–0.5 mg/d in the present study^(20,22,23,25,26,44).

The association between periconception folic acid supplementation and fetal growth could also be explained by optimization of the folate-dependent homocysteine pathway. Reduced folate status is associated with elevated homocysteine and higher serum levels of homocysteine have been associated with decreased fetal growth and placental vasculopathy⁽⁴⁵⁾. In most cases hyperhomocysteinaemia can be treated by low-dose folic acid supplementation. Additionally, it has been shown that folate has the potential to improve endothelial function independent from homocysteine status⁽⁴⁶⁾.

Previous studies showed that folic acid supplementation of 5 mg in mid- and late pregnancy increased placental size, cell number (DNA) and protein content^(21,22). In the present study women who preconceptionally started taking a folic acid supplement did not only have significantly larger newborns but larger placentas as well, compared to women who did not use folic acid. However, this association was not found for women who started folic acid supplementation after pregnancy recognition. The present findings could implicate that periconception low-dose folic acid influenced placental growth via other pathways rather than through improved placental vasculogenesis or its role in placental nucleic acid synthesis.

The results as shown in the present study on periconception folic acid supplementation might suggest that fetal and placental programming are affected by additional folic acid supply at different time frames throughout the periconception period.

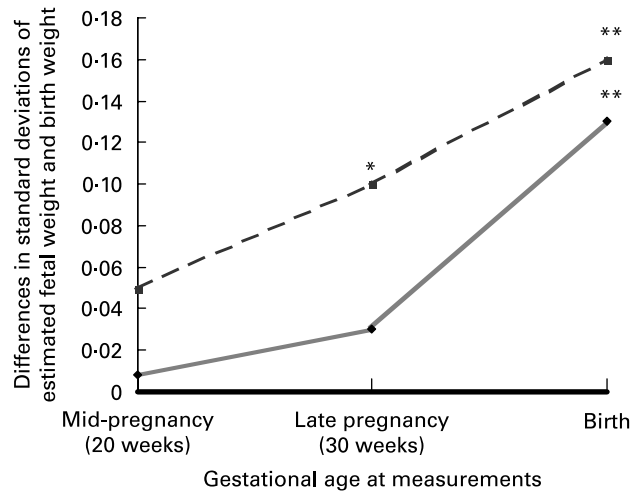


Fig. 1. Associations between periconception folic acid use and estimated fetal weight and birth weight. Results from linear regression analysis. Values are regression coefficients and reflect the difference in standard deviation scores of estimated fetal weight in mid-pregnancy (median 20.5 weeks, 95% range 18.8–23.1) and in late pregnancy (median 30.4 weeks, 95% range 28.5–32.7); and the difference of standard deviations of birth weight, in infants of women who used periconception folic acid supplementation (◆, start before 8 weeks; ■, preconception start), compared to women did not use folic acid (—, reference line). Values are adjusted for gestational age, maternal age, height, weight, parity, ethnicity, fetal gender, educational level and smoking. Values were significantly different from those of the reference (no use) group: * $P < 0.05$, ** $P < 0.001$.

Folate is essential for DNA methylation, an important epigenetic mechanism that plays a regulatory role in genome programming and imprinting during pregnancy^(10,11). Differences in quantitative methylation may affect genes implicated in embryogenesis and fetal growth^(8,46,47). It has been shown that variations in preconception exposure to folic acid can lead to epigenetic modifications of the genome in the offspring associated with adiposity, insulin resistance and high blood pressure in adulthood^(8,29). This might also apply to the present results, suggesting that periconception folic acid supplementation may cause epigenetic modifications in the preimplantation embryo which may result in increased placental and fetal growth patterns^(8,48). However, at this moment these speculations need to be studied in further detail.

An important finding from the present analysis was the modifying effect of parity. The positive effect of parity on birth weight has been well established^(37,38,49,50). However, to our knowledge, the effect modification by parity on the association between periconception folic acid use and fetal growth has not been reported before. In the past, Kloosterman suggested that multiparous women offer, through remodelling

of the maternal vascular structure in former pregnancies, a more favourable environment for placental development and function in subsequent pregnancies^(37,38,49). From this respect it can be hypothesized that periconception folic acid supplementation interacts with these vascular remodelling processes in multiparous women, thereby affecting placental and subsequent fetal growth. However, this needs to be studied further by other investigators.

The present study was embedded in a large prospective cohort study with a significant number of measurements performed in the mothers, which increases the accuracy of our effect estimates. However, some limitations should be addressed. First, because the response rate of the Generation R Study was approximately 61%, selective participation of pregnant women may have influenced the observed associations^(42,51,52). In addition, complete information on folic acid supplementation was missing in approximately 25% of the women, and this non-response could lead to selection bias if the association of periconception folic acid supplementation with fetal growth would differ between those with and without complete data. Even though this seems unlikely, it cannot be fully excluded.

Table 3. Associations between periconception folic acid use, placental weight and placental index* (Regression coefficients and 95% confidence intervals)

	Placental weight				Placental index			
	Model A†		Model B‡		Model A†		Model B‡	
Folic acid supplementation	RC	95% CI	RC	95% CI	RC	95% CI	RC	95% CI
No use	Ref.		Ref.		Ref.		Ref.	
Start before 8 weeks	0.37	–10.20, 10.90	6.84	–4.81, 18.48	–0.003	–0.006, –0.001	–0.001	–0.004, 0.001
Preconception start	10.41	0.32, 20.49	13.28	1.08, 25.48	–0.004	–0.007, –0.002	–0.001	–0.004, 0.002

RC, regression coefficient; Ref., reference.

* For details of subjects and procedures, see Methods and Table 1. Results are from linear regression analysis. Values are regression coefficients and reflect the difference for each characteristic (placental weight in g) compared to no folic acid use.

† Adjusted for gestational age at birth (placental weight).

‡ Adjusted for gestational age at birth (placental weight), maternal age, height, weight, parity, ethnicity, fetal gender, educational level and smoking.

Table 4. Associations between periconception folic acid use and pregnancy complications* (Odds ratios and 95% confidence intervals)

Folic acid supplementation	Low birth weight				SGA				Prematurity			
	Model A†		Model B‡		Model A†		Model B‡		Model A†		Model B‡	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
No use	Ref.		Ref.		Ref.		Ref.		Ref.		Ref.	
Start before 8 weeks	0.75	0.52, 1.07	0.61	0.40, 0.94	0.82	0.53, 1.25	0.69	0.42, 1.14	0.75	0.58, 0.99	0.75	0.55, 1.02
Preconception start	0.47	0.33, 0.68	0.43	0.28, 0.69	0.38	0.23, 0.63	0.40	0.22, 0.72	0.77	0.60, 0.99	0.88	0.63, 1.21

Ref. reference; SGA, small for gestational age.

* For details of subjects and procedures, see Methods and Table 1. Results are from logistic regression analysis.

† Adjusted for gestational age at birth (not prematurity).

‡ Adjusted for gestational age at birth (not prematurity), maternal age, height, weight, parity, ethnicity, fetal gender, educational level and smoking.

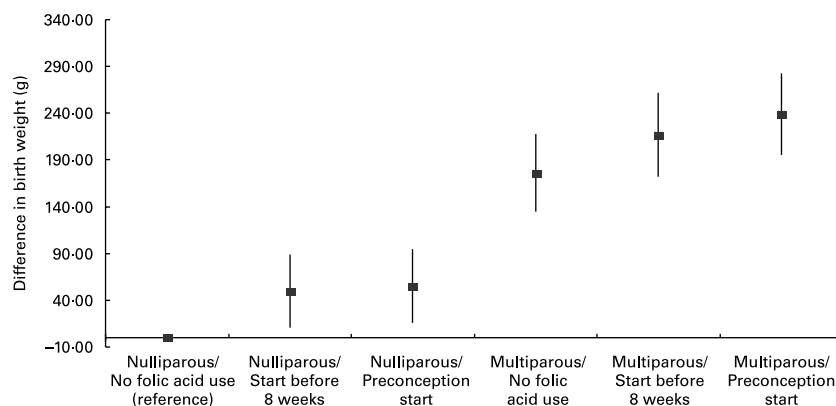


Fig. 2. Associations between periconception folic acid use and birth weight stratified by parity. Results from linear regression analysis stratified per parity/folic acid subgroup. Values are regression coefficients with 95% CI depicted by vertical bars, and reflect the difference in birth weight (in g) compared to nulliparous women who did not use folic acid. All values are adjusted for gestational age at birth, maternal age, height, weight, ethnicity, fetal gender, educational level and smoking.

The use of questionnaires to assess folic acid supplementation encompasses several limitations, including information bias and giving desirable answers. Yet, studies show that self-reported intake of folic acid correlates to folate serum levels⁽⁵³⁾. Moreover, we aimed to assess folic acid supplementation in early pregnancy to minimize possible recall bias. However, even though we used a clear definition of folic acid supplementation in the questionnaire misclassification, especially between those who started using a folic acid supplement preconceptionally and those who started after pregnancy recognition, should always be considered. Last, folic acid supplement use is related to socioeconomic status, lifestyle factors (dietary habits) and adverse health behaviours (smoking). Even though we were able to control for a large number of potential confounders available from our questionnaires, residual confounding is always of particular concern in vitamin supplement studies, and should for this reason be taken into account⁽⁵⁴⁾. For these reasons, the present findings would be much stronger if we had had availability of maternal, placental or neonatal folate biomarkers such as erythrocyte folate levels, or global methylation status.

In conclusion, periconception folic acid supplementation is significantly associated with increased fetal growth resulting in higher placental and birth weight, and decreased risks of having a child with low birth weight or being SGA. The effects are most pronounced in women who preconceptionally start using a folic acid supplement, and are modified by parity.

To investigate the underlying pathways in more detail and possible consequences for postnatal growth and development, future studies are necessary.

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E. A. P. S.; drafting of the manuscript: S. T., V. W. V. J., R. P. M. S.-T.; critical revision of the manuscript for important intellectual content: S. T., V. W. V. J., A. H., R. P. M. S.-T., E. A. P. S.; statistical analysis: S. T., V. W. V. J., R. P. M. S.-T., E. A. P. S.; obtained funding: V. W. V. J., A. H., E. A. P. S.; administrative, technical or material support: A. H., E. A. P. S.; supervision: V. W. V. J., A. H., R. P. M. S.-T., E. A. P. S.

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