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Periconceptional folic acid supplementation and anthropometric measures at birth in a cohort of pregnant women in Valencia, Spain

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Abstract

We examined the relationship between dietary folate intake and periconceptional use of folic acid (FA) supplements, and small-for-gestational age for weight (SGA-W) and height (SGA-H). The study is based on 786 Spanish women aged 16 years or above, who attended the first-term prenatal population-based screening programme (10–13 weeks) at the reference hospital 'La Fe', Valencia, with singleton pregnancy. Periconceptional use of FA supplements was categorised as non-users, moderate users (≤1 mg/d) and high users (>1 mg/d). Babies born to mothers who used high doses of FA supplements had a significant reduction in mean birth height compared with babies of non-users ($\beta = -0{\cdot}53$, 95% CI −0·96, −0·09). As regards weight, mothers using moderate and high doses of FA supplements had lower-birth-weight babies for gestational age than non-users ($\beta = -22{\cdot}96$, 95% CI −101·14, 55·23; $\beta = -89{\cdot}72$, 95% CI −188·64, 9·21, respectively), although these decreases were not significant. Results from the multivariate logistic regression models showed that high FA supplement users had a higher significant risk for SGA-H (OR 5·33, 95% CI 2·08, 13·7), and that users of moderate doses were not associated with a higher risk of either a SGA-W or a SGA-H baby. In contrast, increased quintiles of the dietary intake of folate were associated with a decreased risk of SGA-W (*P* for trend=0·002), although no association was observed for SGA-H. Our findings suggest that periconceptional use of FA supplements greater than 1 mg/d is associated with decreased birth height and may entail a risk of decreased birth weight.

Key words: Pregnancy: Folic acid: Fetal growth: Birth height

The evidence that folic acid (FA) reduces the risk of neural tube defects[1–12] has led many national governments to recommend that women should take 0·4 mg of synthetic FA daily periconceptionally for maintaining a healthy diet[13].

Nowadays, the practice of using FA supplements by pregnant women aims both to prevent congenital malformations and also to correct abnormal folate metabolism or deficiency in order to avoid megaloblastic anaemia and to maintain reproductive health. These have led to distinctively different uses of FA: prenatal; periconceptional; throughout pregnancy[14]. There has been increasing interest in the possible effects of FA supplementation during pregnancy on birth weight, low birth weight (birth weight <2500 g), pre-term delivery (<37 weeks) and small-for-gestational age (SGA).

Overall, epidemiological studies have suggested that adequate FA during pregnancy promotes fetal growth[15–21]. This has been supported by a population-based study of 6 million Californian births, where small but significant risk reductions were reported for low birth weight (6%) and pre-term delivery (4%) before and after mandatory FA fortification[22]. However, other observational studies have not found such associations[23–25], and a meta-analysis of twenty-two randomised controlled trials found no evidence that FA supplementation after the first trimester had any beneficial effect on mean birth weight and gestational age at delivery. The authors concluded that periconceptional folate use should not be continued through pregnancy[26]. To our knowledge, only two epidemiological studies have specifically studied the relationship between periconceptional use of FA and fetal growth. One double-blind randomised trial showed that preconception use of daily doses of FA supplements increased birth weight and decreased SGA only in babies

Abbreviations: FA, folic acid; SGA, small-for-gestational age; SGA-H, small-for-gestational age for height; SGA-W, small-for-gestational age for weight.

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born after week 42 of pregnancy compared with the non-supplemented group[27]. In a population-based cohort study, periconceptional use of a low dose of FA supplements was associated with a decreased risk of low birth weight and SGA compared with non-users. The greatest effect was observed on newborns from mothers who started supplementation before conception. Their babies had 68 g higher birth weight compared with the babies of non-users[28].

The lack of consistency among studies makes it difficult to draw conclusions about whether or not FA supplementation influences fetal growth. Although folate is safe and almost free of toxicity[13], several studies have drawn attention to the possible side effects of using higher doses of synthetic FA than those recommended[29,30]. An improvement in the periconceptional use of FA supplements by Spanish pregnant women has been reported in recent years. However, most of these women are apparently using much higher FA supplement doses than recommended[31,32].

The aim of the present study was to examine the effect of periconceptional FA supplementation use and dietary folate intake on birth weight, small-for-gestational age for weight (SGA-W) and height (SGA-H) in a cohort study of pregnant women in Valencia, Spain.

Methods

Population and study design

The present investigation is part of the Spanish population-based multi-centre cohort study, 'Infancia y Medio Ambiente', which aims to examine the role of diet and environmental factors on fetal growth and infant health until young adulthood in several Spanish cities. The mothers' recruitment and follow-up procedures are described elsewhere[33–35]. In brief, the Spanish health care system includes a standardised and regular screening programme for congenital malformations between the 10th and 13th weeks of gestation. Pregnant women from a well-defined geographic area in the Valencia region were recruited when they attended their first prenatal visit in La Fe Hospital. Women were eligible if they fulfilled the following inclusion criteria: age 16 years or above; singleton pregnancy; no assisted conception; inclusion visit at 10–13 weeks; delivery foreseen at the reference hospital; no communication handicap. From February 2004 to June 2005, 840 of 1563 eligible women were included in the study (54 % participation rate). Participants were slightly older with similar educational levels, but they were more likely to be working than non-participants. The final analyses included 787 women who gave birth to a singleton live infant between May 2004 and February 2006.

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and the study protocols were approved by the Ethics Committee of La Fe Hospital (Valencia, Spain).

Small-for-gestational age

Information on anthropometric measures at birth was obtained from the hospital delivery logs. After birth, the midwife measured birth weight, and birth height was measured during the first 12 h. Gestational age was established based on the last menstruation period. When the difference between the last menstruation period and the first-trimester ultrasound was equal to or greater than 7 d, gestational age was established by ultrasound. In 12·5 % of the women, a first-trimester ultrasound of the crown–rump length was used to date gestational age. Birth weight and height were adjusted for sex to correspond to a gestational age of 40 weeks using the residual method. We defined SGA-W or SGA-H as that weight or length below the 10th percentile based on Spanish standardised growth reference charts for both sexes[36]. Neither SGA-W nor SGA-H could be defined for a newborn whose gestational age (23 weeks) had no percentile value. Pre-term births were defined as babies born at less than 37 weeks. Consequently, the final sample was composed of 786 women who gave birth to a live singleton baby.

Periconceptional folic acid use and folate intake

During their first visit, women were asked the following question: 'In the 3 months prior to becoming pregnant and up to the present, have you taken FA or multivitamins?' Information regarding the date supplement use began, the frequency of use (times/week), dose and brand names of supplements was also collected.

Periconceptional use of FA supplements was defined as any use involving supplements containing FA alone and/or FA from multivitamin supplements, from 3 months before conception up to and including the first month of pregnancy. Women who took FA supplements daily for at least 1 month during the studied period were defined as users of periconceptional FA. Those who never used supplements containing FA during periconception were defined as non-users. Thus, FA supplement users were categorised into moderate users ($\leq$1 mg/d, ranging from 0·2 to 0·9 mg/d) and high users ($>$1 mg/d, ranging from 2·5 to 10·5 mg/d). Only 5·2 % of FA supplement users received it in the form of multivitamin supplements.

Usual dietary folate intake was measured using a modified version of the Harvard FFQ[37], previously adapted and validated for a general adult Spanish population[38,39]. The FFQ had 100 food items to assess usual food and nutrient intakes during the first trimester of pregnancy. Caffeine (mg/d) and alcohol (g/d) intakes were also assessed in the FFQ. Nutrient values were obtained from the US Department of Agriculture food composition tables and other published sources[40]. We asked how often, on average, participants had consumed a particular amount of a specific type of food from the last menstrual period until the time of the interview. We calculated nutrient intakes by multiplying the frequency of consumption for each food item by the nutrient composition of the portion size specified on the FFQ and by adding the total across all foods to obtain a total nutrient intake for each individual. We used the residual method to estimate energy-adjusted values for nutrient intakes[37].

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Covariates

Information on covariates was obtained from questionnaires completed at personal interview. Covariates considered were maternal age at entry in the study (≤29, 30–34 and ≥35 years), years of education (≤primary school, secondary school and university), country of origin (Spain and other countries), lifestyle variables such as current smoking (yes or no), alcohol consumption (g/d), hours of television viewing during the first trimester and paternal height (in cm) as stated by the mother. Relevant data about past and present medical and obstetric history, including complications relating to the current pregnancy such as gestational hypertension, gestational diabetes, pre-pregnancy BMI, gestational weight gain, planned pregnancy, attendance by private gynaecologist and parity, were also collected. The variable 'medical problems in previous pregnancies' was derived from the information on previous obstetric history, including instances of miscarriage, stillborn offspring, previous pre-term and low-birth-weight births and congenital malformations. We collected information on chronic diseases such as diabetes, high blood pressure, CVD, and allergies, drug use, family medical conditions and vaccinations during pregnancy. Pre-pregnancy BMI was calculated by dividing self-reported weight before pregnancy (in kg) by the square of the self-reported height (in m). Total gestational weight gain was calculated as the product of gestational length (in weeks) and weekly maternal weight gain. The latter was previously adjusted for the last available gestational age to avoid the non-linearity of weight gain during pregnancy. Both BMI and weight gain were further classified following the guidelines of the Institute of Medicine[41]. As such, total weight gain was classified according to the recommended total weight-gain ranges for pregnant women, based on pre-pregnancy BMI, as follows: low, from 12·5 to 18 kg for women with a pre-pregnancy BMI $<19{\cdot}8\,kg/m^2$; normal, from 11·5 to 16 kg, for pre-pregnancy BMI of $19{\cdot}8–26{\cdot}0\,kg/m^2$; high, from 7 to 11·5 kg for pre-pregnancy BMI $>26{\cdot}0–29{\cdot}0\,kg/m^2$. For example, a woman with a pre-pregnancy BMI $<19{\cdot}8\,kg/m^2$ and total weight gain higher than 18 kg was classified as having a high total weight gain.

Statistical analysis

For descriptive purposes, characteristics of periconceptional FA users and non-users were expressed as percentages or means and standard deviations or medians (interquartile ranges). Dietary folate data were treated logarithmically to take into account their skewed distributions and adjusted according to the total energy intake model described by Willett *et al.*[37]. These standardised values were then categorised into quintiles, with the lowest quintile treated as the reference category. We performed bivariate analyses to determine maternal and infant characteristics associated with periconceptional FA use. ANOVA or the Kruskal–Wallis tests were used to compare means of continuous variables and χ^2 tests for categorical variables. Height of both parents, dietary intake of FA, alcohol consumption and hours of television viewing were included as continuous variables, as they showed a linear relationship.

Logistic regression analysis was used to estimate the independent effect of periconceptional use of FA on the risk of SGA-W (model 1) and SGA-H (model 2), where the reference category was non-users of supplements. We included the FA supplement use and quintiles of dietary folate, together with all potential confounding factors in the same model. We used the likelihood ratio test for the exclusion or inclusion of covariates. All covariates with P values $<0{\cdot}20$ or those reported in the biomedical literature as determinants of fetal growth were retained in the model. Covariates were considered confounders if they changed the estimation of FA use related to either SGA-W or SGA-H by more than 10 %. Models were adjusted for maternal age, country of origin, education, dietary intake of FA, gestational diabetes and hypertension, gestational weight gain, parity, planned pregnancy, history of medical problems in previous pregnancies, private gynaecologist and maternal height and paternal height. A P value $<0{\cdot}05$ was considered as significant for the estimate of the effect.

Multiple linear regression analysis was used to estimate the independent associations between continuous birth outcomes (BW and BH) and the use of FA supplements, adjusting for covariates following the same strategy as for dichotomous birth outcomes.

Results

In the present study, 30 % of women reported daily periconceptional use of FA supplements (either moderate doses, mean 331 (SD 130) μg/d, or high doses, mean 4560 (SD 2013) μg/d; $P<001$; Table 1). FA supplement users were slightly older, better educated, mainly Spanish, more likely to be nulliparous, more likely to have planned the pregnancy, more often attended by a private gynaecologist, more likely to give birth to babies with a lower birth weight and height and had more SGA-W and SGA-H babies than non-users. Users and non-users were similar in alcohol consumption, smoking, hours of television viewing, prevalence of gestational diabetes and hypertension, and dietary intake of folate.

Mean birth weight and height of babies adjusted for gestational age and sex were lowest for women using high doses of supplements (3324 (SD 432) g and 50·2 (SD 2·2) cm, respectively) compared with those for moderate dose users and non-users (Table 2). However, increasing quintiles of dietary folate were associated with higher birth weight and height, although mean weight and mean height for the fourth quartile were somewhat lower than those for the third quintile. β-Coefficients from the multiple linear regression analysis showed that babies of mothers who used high doses of FA supplements had a significant reduction in mean birth height of 0·5 cm compared with babies of non-users ($\beta=-0{\cdot}53$, 95 % CI −0·96, −0·09). As regards weight, mothers using moderate and high doses of FA supplements had lower-birth-weight babies for gestational age than non-users ($\beta=-22{\cdot}96$, 95 % CI −101·14, 55·23; $\beta=-89{\cdot}72$, 95 % CI −188·64, 9·21, respectively), although these decreases were not significant.

Table 1. Characteristics of pregnant women (*n* 786) and their newborns of the Infancia y Medio Ambiente-Valencia cohort study according to the periconceptional use of folic acid (FA) supplements by doses (Mean values, standard deviations, *n* and percentages)

	FA supplement use												
	Non-users (*n* 550)				Moderate (≤ 1 mg/d, *n* 152)				High (> 1 mg/d, *n* 84)				
	Mean	SD	*n*	%	Mean	SD	*n*	%	Mean	SD	*n*	%	*P**
Demographics and lifestyles													
Maternal age (years)													
≤29			258	46·9			74	48·7			28	33·3	0·140
30–34			206	37·5			53	34·9			42	50·0	
> 35			86	15·6			25	16·4			14	16·7	
Education													
≤ Primary school			202	36·7			51	33·6			14	16·7	<0·001
Secondary school			241	43·8			52	34·2			42	50·0	
University			107	19·5			49	32·2			28	33·3	
Country of origin													
Spain			470	85·5			141	92·8			83	98·8	<0·001
Other countries			80	14·5			11	7·2			1	1·2	
Smoking – first trimester, % smokers (missing = 7)			145	26·6			33	21·9			16	19·0	0·208
Alcohol – first trimester, % non-drinkers (missing = 5)			371	68·1			111	73·0			62	73·8	0·342
TV watching (h/d) (missing = 2)			2·4	1·5			2·4	1·5			2·1	1·5	0·195
Pregnancy characteristics													
Gestational diabetes			31	5·6			9	5·9			2	2·4	0·438
Gestational hypertension			16	2·9			8	5·3			2	2·4	0·314
Gestational wt gain (missing = 9)													
Normal			197	36·3			60	40·0			30	35·7	0·152
Low			129	23·8			44	29·3			16	19·0	
High			217	40·0			46	30·7			38	45·2	
Planned pregnancy (missing = 9)			376	69·2			138	91·4			75	90·4	<0·001
Private gynaecologist			165	30·0			61	40·1			41	48·8	0·001
Maternal ht (cm)	161·8	6·3			161·8	6·7			164·0	6·0			0·011
Paternal ht (cm)	175·0	7·2			176·4	7·5			177·4	6·5			0·005
Obstetric history													
Parity													
0			285	51·8			95	62·5			54	64·3	0·013
≥ 1			265	48·2			57	37·5			30	35·7	
Medical problems in previous pregnancies†			103	18·7			37	24·3			22	26·2	0·130
Newborn characteristics													
Birth wt‡	3344	417			3282	445			3324	432			0·153
Birth ht‡	50·6	1·8			50·3	1·8			50·2	2·2			0·041
SGA-W§			52	9·5			24	15·8			14	16·7	0·027
SGA-H§			25	4·6			11	7·2			9	10·7	0·052
Gestational age	39·6	1·9			39·3	1·6			39·6	1·8			0·164
Sex (newborn)													
Female			257	46·7			76	50·0			37	44·0	0·651
Male			293	53·3			76	50·0			47	56·0	
Folic acid status													
Dietary folate intake (μg/d) (missing = 5)	305	102			295	92			315	100			0·324
Energy intake (kJ/d) (missing = 5)	9673·41	2510·4			9526·97	2380·69			9439·10	2250·99			0·634
Folic acid intake from supplements (μg/d)	0				331	130			4560	2013			<0·001

TV, television; SGA-W, small-for-gestational age for weight; SGA-H, small-for-gestational age for height.
* *P* value from the χ^2 test and ANOVA.
† Spontaneous abortion, stillborn offspring, previous pre-term and low-birth-weigh births and congenital malformations.
‡ Birth weight and height standardised for sex and gestational age using the residual method.
§ Newborns whose weight and/or height were below the 10th percentile according to the Spanish growth reference charts.

Table 2. Multiple linear regression between periconceptional use of folic acid (FA) supplements and weight and height at birth adjusted for gestational age in the Infancia y Medio Ambiente-Valencia cohort study, 2004–6 (Mean values, standard deviations, β and 95 % confidence intervals)

	Total	Mean	SD	β*	95 % CI
Birth wt†					
FA supplement use					
Non-users	550	3344	417		–
Moderate ≤ 1 mg/d	152	3282	445	−22·96	−101·14, 55·23
High >1 mg	84	3324	432	−89·72	−188·64, 9·21
P‡	0·087				
Quintiles of dietary folate intake§					
≤211·1	155	3207	402		–
211·2–245·4	157	3306	449	98·96	6·37, 191·56
245·5–283·7	156	3383	424	155·07	60·94, 249·20
283·8–332·0	157	3338	389	126·88	32·38, 221·38
> 332	156	3388	468	168·50	72·62, 264·39
P‡	0·001				
Birth ht†					
FA supplement use					
Non-users	550	50·6	1·8		–
Moderate ≤1 mg/d	152	50·3	1·8	−0·24	−0·59, 0·10
High >1 mg	84	50·2	2·2	−0·53	−0·96, −0·09
P‡	0·012				
Quintiles of dietary folate intake§					
≤211·1	155	50·3	1·7		–
211·2–245·4	157	50·4	2·1	−0·02	−0·39, 0·43
245·5–283·7	156	50·8	1·91	−0·40	−0·02, −0·81
283·8–332·0	157	50·6	1·6	0·23	−0·18, 0·81
> 332	156	50·7	1·8	0·29	−0·13, 0·71
P‡	0·108				

* β-Coefficients and 95 % CI from multiple regression adjusted for maternal age (≤29, 30–34 and ≥35 years), country of origin (Spain and other countries), education (≤primary school, secondary school and university), energy intake (kJ), smoking (no or yes), alcohol intake (no or yes), television viewing (h/d), gestational diabetes (no or yes), transient hypertension (no or yes), gestational weight gain (normal, low and high), parity (0 and ≥1), planned pregnancy (no or yes), history of medical problems in previous pregnancies (no or yes), private gynaecologist (no or yes), maternal height (cm) and paternal height (cm).
† Birth weight (g) and height (cm) adjusted for gestational age and sex.
‡ *P* value for linear trends examined using likelihood ratio tests.
§ Quintiles of dietary folate intake (μg/d) adjusted by total energy intake.

Similarly, results from the multivariate logistic regression models (Table 3) showed that high FA supplement users showed a higher significant risk for SGA-H (OR 5·33; 95 % CI 2·08, 13·7) than non-users. An association for SGA-W (OR 2·05, 95 % CI 0·98, 4·28), which was not-significant, was also observed. On the other hand, users of moderate doses were not associated with a higher risk of either a SGA-W or a SGA-H baby, whereas increasing quintiles of dietary folate were associated with decreasing risk for SGA-W, with maximum protection from intakes above 245·4 μg/d (*P* for trend=0·002). However, no association was observed for SGA-H. The logistic regression model for height (Table 3) was repeated using fetal growth restriction for height as the dependent variable instead of SGA-H (results not shown). Briefly, fetal growth restriction was estimated based on constitutional growth potential to differ growth-restricted newborns from constitutionally small ones. To define fetal growth restriction, we predicted each anthropometric measure at birth (i.e. birth height) using a customised model, taking into account parental (maternal preconception weight, height, and parity and paternal height) and newborn variables (sex and gestational age). Subsequently, we classified fetal growth restriction as those newborns below the 10th percentile of the predicted birth-height distribution[41]. It was observed that although the effect of high doses of FA supplements on fetal growth restriction for length was smaller compared with the effect on SGA-H, this effect was still strong and statistically significant (OR 3·18, 95 % CI 1·47, 6·86; OR 5·33, 95 % CI 2·08, 13·7, respectively).

Discussion

The present study found that pregnant women who used high periconceptional doses of FA supplements had a significantly higher risk of having a SGA-H baby. Those mothers taking moderate periconceptional doses of FA supplements were not at risk of having either a SGA-W or a SGA-H baby. In contrast, increasing quintiles of dietary folate showed a significant reduction in the risk of SGA-W, but no association was found for SGA-H.

Increased risk of SGA with the intake of high doses of FA supplements has not been previously reported, either when examining the use of FA supplements during the whole pregnancy[19–21,23,24,26] or when studying the periconceptional use of FA on fetal growth[27,28]. In the present study, the associations between dietary folate intake and high doses of supplements of FA with birth anthropometry were unexpected and opposite. While dietary folate is the naturally occurring water-soluble vitamin B_9 form found in fresh foods, FA is the synthetic oxidised form, which must be converted to

Table 3. Multiple logistic regression analysis between the periconceptional use of folic acid (FA) supplements and the risk of small-for-gestational age for weight and height (SGA-W and SGA-H) in the Infancia y Medio Ambiente-Valencia cohort study, 2004–2006

(*n*, percentages, odds ratios and 95 % confidence intervals)

		SGA*				
	Total	*n*	%	OR adjusted†	95 % CI	*P*‡
Model 1						
SGA-W*						
FA supplement use						
Non-users	550	52	9·5	1·00		0·047
Moderate ≤ 1 mg/d	152	24	15·8	1·38	0·76, 2·51	
High > 1 mg/d	84	14	16·7	2·05	0·98, 4·28	
Quintiles of dietary folate intake§						
≤ 211·1	155	26	16·8	1·00		0·002
211·2–245·4	157	23	14·6	0·86	0·44, 1·68	
245·5–283·7	156	13	8·3	0·39	0·18, 0·85	
283·8–332·0	157	9	6·4	0·21	0·09, 0·50	
> 332	156	18	11·5	0·48	0·22, 1·02	
Model 2						
SGA-H*						
FA supplement use						
Non-users	550	25	4·6	1·00		0·001
Moderate ≤ 1 mg/d	152	11	7·2	1·93	0·84, 4·44	
High > 1 mg/d	84	9	10·7	5·33	2·08, 13·7	
Quintiles of dietary folate intake§						
≤ 211·1	155	10	6·5	1·00		0·110
211·2–245·4	157	13	8·3	1·33	0·53, 3·33	
245·5–283·7	156	9	5·8	1·00	0·37, 2·72	
283·8–332·0	157	9	5·7	0·96	0·35, 2·68	
> 332	156	4	2·6	0·35	0·10, 1·24	

* Newborns whose weight and/or height were below the 10th percentile according to the Spanish growth reference charts.

† OR adjusted for maternal age (≤29, 30–34 and ≥35 years), country of origin (Spain and other countries), education (≤primary school, secondary school and university), energy intake (kJ), smoking (no or yes), alcohol intake (no or yes), television viewing (h/d), gestational diabetes (no or yes), transient hypertension (no or yes), gestational weight gain (normal, low and high), parity (0 and ≥1), planned pregnancy (no or yes), history of medical problems in previous pregnancies (no or yes), private gynaecologist (no or yes), maternal height (cm) and paternal height (cm).

‡ *P* value for linear trends examined using likelihood ratio tests.

§ Quintiles of dietary folate intake (μg/d) adjusted by total energy intake.

tetrahydrofolate by dihydrofolate reductase in the human liver to be biologically active[42]. The activity of dihydrofolate reductase becomes saturated when FA is consumed at levels higher than the tolerable upper intake level for FA (1 mg/d for adults), leading to a considerably increased exposure to unmetabolised FA. In fact, it seems that doses above 0·4 mg/d result in the appearance of unmetabolised FA[43]. Concerns have been expressed that high doses of FA supplements (>1 mg/d) and the presence of plasma unmetabolised FA may exacerbate some pre-existing cancers or the progression of precancerous lesions[44,45]. Methyl donors such as FA are essential for the methylation of DNA required to regulate gene expression, transcription and stability of DNA. It has been suggested that increased methylation of insulin-like growth factor 2-imprinted gene is associated with decreased birth weight, since it may play a central role in matching the placental nutrient supply to the fetal nutrient demands for growth[42,46]. The mechanism underlying the adverse effects of the use of high doses of FA, unmetabolised circulating FA and reproductive health is unclear, but it seems that methyl donors can have a profound impact upon reproductive health through an epigenetic mechanism[47].

The prevalence of preconceptional use of FA supplements in the present study (18·8 %) is similar to the latest trends found for Spanish pregnant women (17·37 %) reported for the period 1991–2004[32]. However, the prevalent use of high doses of FA supplements (1–5 mg/d) has been found to be higher (57·01 %) than was observed in the present study (36·9 %). Given these trends on high dose supplementation practices, if the associations observed in the present study are proved true, one would expect a greater effect of periconceptional FA supplements on small-for-gestational age at the population level. On the other hand, we found that pregnant women with the highest dietary intake of folate (>245 μg/d) had a 60 % decreased risk of having SGA-W babies compared with women with the lowest folate intake (first quintile <211 μg/d), and this was independent of FA supplement use. In fact, high vegetable intake (the main food source of folate) was found to be significantly associated with higher weight and length at birth in our population[35]. Similar results were found in a prospective US study showing that women with the lowest FA intake (≤240 μg/d) had a twofold risk of having a low-birth-weight baby[18].

Reverse causality might explain our findings. There was no specific question asking why women in the present study used the FA supplements. To explore whether reverse causation or potential indication bias might have occurred (either self-selected use of FA or prescribed by health professionals), we

repeated the statistical analysis excluding the 162 women who reported any medical complication in previous pregnancies. Results remained unchanged, and consequently we believe that reverse causality might not have occurred.

Previous studies have shown that maternal exposure to FA antagonists in the 1-year period before delivery, such as anti-epileptic drugs, drugs used in mood disorders, in urinary tract infections and in CVD might be associated with fetal growth restriction[48]. In the present study, there was no information about medication use in the year before delivery, but the relationship between the presence of chronic diseases including CVD, depression, urinary tract infection, Crohn's disease and other infections during pregnancy and SGA was examined. No association was observed between any of these diseases and SGA. Besides the possibility of residual confounding, several other potential limitations may have affected our findings. The 54 % participation rate may have affected the external validity of the present study, but generalising the results to the whole population of pregnant women in the Valencia region was beyond the scope of the present study. Selective participation of pregnant women may not be ruled out completely if the decision to participate had been related to the risk factors under study. In cohort studies, the decision to participate cannot be based upon future outcomes[49], but the decision to participate may correlate with social, educational and health conditions, which may be associated with risk factors for birth outcomes[50]. In the present study, participants and non-participants had similar various sociodemographic characteristics except for working status and age, with a higher proportion of working women and slightly higher mean age among participants (mean age 30·2 *v.* 29·8 years, respectively). The use of a questionnaire to assess FA supplementation may have led to the misclassification of exposure, despite the clear question concerning supplements. We were unable to validate self-reported FA supplement use against serum folate levels, a more reliable index of human folate status. The fact that we asked for FA supplementation use in early pregnancy may have minimised a possible recall bias. Due to the prospective design, self-reported supplement use was not influenced by the newborn birth weight or height, and if there was a misclassification, it is likely to have been non-differential. Finally, the use of an FFQ may have led to the misclassification of dietary folate intake. Folate was not key to the design of the present FFQ, but results from the validation study carried out within the same population of pregnant women showed good correlation coefficient between total folate intake and serum folate in the third month of pregnancy (r 0·50, $P<0·01$; J Vioque, EM Navarrete-Muñoz, D Gimenez, M Rebagliato, F Ballester, R Ramón, M Murcia, M García de la Hera and C Iñiguez, unpublished results). The main food sources of folate in our questionnaire were presented either as separate food items (wheat bread, whole wheat bread, bananas, oranges and spinach) or in food groups with similar folate content (broccoli, Brussels sprouts and cabbage, or lettuce, endive or chicory). For these reasons, and despite its limitations, we believe that the FFQ used in the present study was adequate to discriminate high from low consumers of folate intake.

The present study has several strengths. It has a prospective design with many high-quality measurements performed on mothers and newborns, which increases the accuracy of our effect estimates. Trained interviewers prospectively collected the data, and few participants were lost to follow-up (4 %), providing internal validity to our findings. The effect estimates persisted after adjustment for all the confounders identified along with other important determinants of birth weight and height.

In conclusion, it has yet to be elucidated whether FA might be related to DNA methylation changes and decreased fetal growth. Although observational studies cannot prove metabolic mechanisms, a randomised controlled trial is neither ethical nor feasible. Given the current increasing Spanish trends regarding high doses of FA supplements, large population-based cohort studies might be one of the few ways to investigate this question further. These studies should include detailed information on FA supplement use, such as medical indication or self-supplementation use, and examine the effect of FA supplements on DNA methylation.

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