

Original Investigation

Folic Acid Supplements During Pregnancy and Child Psychomotor Development After the First Year of Life

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IMPORTANCE Folate intake during pregnancy has been associated with improved neuropsychological development in children, although the effects of high dosages of folic acid (FA) supplements are unclear.

OBJECTIVE To examine the association between the use of high dosages of FA supplements during pregnancy and child neuropsychological development after the first year of life.

DESIGN, SETTING, AND PATIENTS The multicenter prospective mother-child cohort Infancia y Medio Ambiente (INMA) Project recruited pregnant women from 4 areas of Spain (Asturias, Sabadell, Gipuzkoa, and Valencia) between November 2003 and January 2008. Pregnant women completed an interviewer-administered questionnaire on the usual dietary folate intake and FA supplements at 10 to 13 weeks and 28 to 32 weeks of gestation. The main analyses were based on a sample of 2213 children with complete information on neuropsychological development and FA supplement intake during pregnancy. Multiple linear and logistic regression analyses were used to explore the effects of FA supplements on child neuropsychological development.

MAIN OUTCOMES AND MEASURES Neuropsychological development was assessed using the Bayley Scales of Infant Development. We calculated mental scale and psychomotor scale scores. One SD below the mean established a delay in neurodevelopment (score <85).

RESULTS A high proportion of women (57.3%) did not reach the recommended dosages of FA supplements (400 µg/d), but 25.2% women took more than 1000 µg/d of FA supplements (3.5% consuming >5000 µg/d). In multivariate analysis, we observed that children whose mothers used FA supplement dosages higher than 5000 µg/d during pregnancy had a statistically significantly lower mean psychomotor scale score (difference, -4.35 points; 95% CI, -8.34 to -0.36) than children whose mothers used a recommended dosage of FA supplements (400-1000 µg/d). An increased risk of delayed psychomotor development (psychomotor scale score <85) was also evident among children whose mothers took FA supplement dosages higher than 5000 µg/d, although the association was not statistically significant (odds ratio = 1.59; 95% CI, 0.82-3.08).

CONCLUSIONS AND RELEVANCE To our knowledge, this is the first time a detrimental effect of high dosages of FA supplements during pregnancy on psychomotor development after the first year of life has been shown. Further research from longitudinal studies is warranted to confirm these results.

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Folic acid (FA) or folate (natural form) is an essential micronutrient in the B-complex vitamins. It is involved in different physiological processes, particularly growth and fetal development because of its role in the synthesis, repair, and methylation of DNA, contributing to the formation of new cells and tissues.¹ The nutritional demands for FA increase during pregnancy, a period of rapid growth. The direct relationship between FA deficiency in pregnant women and the occurrence of birth defects in the neural tube has been widely documented,¹ and recommendations have been established by health institutions all over the world. Thus, in addition to an appropriate dietary folate intake, the periconceptional use of FA from supplements at a dosage of 400 µg/d, not exceeding the tolerable upper limit of 1000 µg/d, is recommended to prevent neural tube defects.²

Some nutrients have a major influence on formation of the brain and development of the nervous system.³⁻⁵ Among them, FA plays an important role in the proliferation and growth of glial and neuronal cells and in the synthesis of neurotransmitters.⁴ Despite the involvement of FA in neural development and its role in preventing the risk of neural tube defects, the number of epidemiological studies exploring the association between FA use during pregnancy and neuropsychological development is low. Moreover, the results are not fully consistent, probably as a consequence of the different criteria used regarding child age, timing and dosages of FA supplement use, and other factors.⁶⁻¹⁹ Animal studies have shown that folate availability may affect brain development long after neural tube closure and that FA supplement use during pregnancy is related to better neuropsychological development and neurodevelopment of offspring.^{20,21} However, many questions remain on the use of moderate or high FA supplement dosages, the frequency and timing of FA supplement use during pregnancy, and their potential effects on child neurodevelopment.

Most of the human studies have evaluated children at school age (≥ 3 years) and found a positive association between the use of FA supplements and child neuropsychological development.⁶⁻¹⁷ The majority of studies collected information on FA supplement use in early pregnancy; only 5 studies collected this information during the entire pregnancy.^{10,14-16,19} A low number of studies assessed neuropsychological development in early childhood, ie, younger than 18 months.^{9,13,17} Only 2 studies included children at ages 2 and 6 years, and they did not find a beneficial effect of FA supplements on neuropsychological development.^{18,19} It is also significant that the dosages of FA supplements used by pregnant women were very different among studies.

Thus, current evidence makes it difficult to reach conclusions on whether the use of FA supplements may benefit child neurodevelopment. Although FA is safe and almost free from toxic effects,²² several studies, including experimental animal investigations, have drawn attention to the possible detrimental effect of using FA supplement dosages higher than those recommended.²³⁻²⁶ To date, it remains unclear how FA supplements may affect child neurodevelopment after birth. Therefore, the aim of this study was to examine the association between the use of high dosages of FA supplements during pregnancy and child neurodevelopment at age 1 year.

Methods

Study Design and Population

Population-based birth cohorts were established as part of the Infancia y Medio Ambiente (INMA) Project, a multicenter cohort study that aims to investigate the effect of environmental exposures and diet during pregnancy on fetal and child development in different geographic areas of Spain (<http://www.proyectoinma.org>). This multicenter mother and child cohort study was set up in Spain between November 2003 and January 2008 in the regions of Valencia, Sabadell (Catalonia), Asturias, and Gipuzkoa (Basque Country) with a common protocol.²⁷

Women were enrolled during the first antenatal care visit to the main public hospital or health center of reference and were followed up through pregnancy. A total of 2644 women agreed to participate. Excluding the women who withdrew from the study, were lost to follow-up, had induced or spontaneous abortions, or had fetal deaths, a total sample of 2506 women delivered a live infant between May 2004 and August 2008. Of these infants, 2226 (88.5%) were evaluated for neuropsychological development at 1 year of age (February 2006 to October 2009). Parents provided written informed consent, and the research protocol was approved by the ethics committees of the centers involved in the study (Hospital La Fe, Valencia; Hospital de Cruces, Gipuzkoa; Hospital de Sabadell, Sabadell; and Hospital Universitario Central de Oviedo, Asturias).

Neuropsychological Assessment

The cognitive and psychomotor development of infants was evaluated through the first edition of the Bayley Scales of Infant Development (BSID).²⁸ The tests were administered by trained psychologists at a mean age of 14.8 months (range, 11.3-22.9 months). Thirteen children older than 23 months at testing were excluded from analysis, leaving a final sample of 2213 children. The mental scale (MS) consisted of 163 items that assessed cognitive development in areas such as performance ability, memory, and first verbal learning. The psychomotor scale (PS) comprised 81 items assessing fine and gross psychomotor development. Special conditions at the time of testing that could affect the quality of the BSID evaluation were registered (eg, rejecting behaviors, irritability, tiredness, sleepiness, fever). The BSID scores were standardized taking into account the psychologist who administered the test and the child's age at evaluation, using parametric methods for the estimation of age-specific reference intervals.²⁹ Standardized residuals were typified to a mean of 100 with an SD of 15 points to homogenize the scales. One SD below the mean was used to identify a delay in neuropsychological development (score < 85). Dichotomous variables were created for both the MS and the PS to identify children at risk for delayed neuropsychological development.

To limit interobserver variability, we applied a strict protocol, including interobserver trainings and 3 sets of quality controls (interobserver reliability tests) undertaken during the fieldwork. The interrater reliability was estimated by intra-

class correlation with coefficients of 0.90 for the MS and 0.96 for the PS. Furthermore, Cronbach α was used to determine the internal consistency of each of the scales. A good coefficient would be a value of 0.70 or higher³⁰; the α coefficient was 0.70 for the MS and 0.73 for the PS.

Assessment of Usual Dietary Folate Intake and FA Supplements

Usual dietary folate intake was calculated averaging estimates from two 100-item, semiquantitative food frequency questionnaires (FFQs), administered in personal interviews at 2 points in pregnancy: at 10 to 13 weeks to estimate FA intake from preconception to 10 to 13 weeks, and at 28 to 32 weeks to estimate FA intake in the second period of pregnancy (from 10-13 weeks to 28-32 weeks). The FFQ was an adapted version of the questionnaire by Willett et al,³¹ validated and developed for Spanish pregnant women in Valencia.³² Folate content of the food items was primarily obtained from food composition tables from the US Department of Agriculture³³ and other published sources.³⁴ We calculated nutrient intakes by multiplying the frequency of use for each food item by the nutrient content of the portion size specified on the FFQ and then adding across all foods to obtain a total nutrient intake for each individual. We used the residual method to estimate calorie-adjusted values for nutrient intakes.³⁵ Once the usual daily dietary intake of FA was estimated for each woman in each period, we averaged both periods and classified women according to tertiles of daily intake (120-271, 271-326, and 326-684 $\mu\text{g}/\text{d}$).

The consumption of FA supplements or vitamin/mineral preparations containing FA was collected using a structured questionnaire and additional questions based on supplement brand names and composition, daily dose, and timing of consumption. With this information, FA supplement use was calculated from 3 months before conception to the seventh month of pregnancy. The results from the validation study carried out within the same population of pregnant women showed a Pearson correlation coefficient of 0.12 between dietary folate intake and serum folate level in the third month of pregnancy. When FA supplement use was considered, the Pearson correlation coefficient between FA total intake and serum folate level was 0.53.³² Intake of FA supplements during the months of consumption was calculated as the mean of FA supplement intake, classifying the women into the categories with use of less than 400 $\mu\text{g}/\text{d}$, 400 to 1000 $\mu\text{g}/\text{d}$, 1000 to 5000 $\mu\text{g}/\text{d}$, and more than 5000 $\mu\text{g}/\text{d}$.

Other Covariates

Questionnaires administered by personal interview in the first and third trimesters were used to collect maternal characteristics: mother's age (in years), country of origin (Spain or other), educational level (primary/none, secondary, or university), social class (I/II [high], III, or IV/V [low]), planned pregnancy (yes or no), parity (0 or ≥ 1 previous child), and smoking during pregnancy (no or yes). Previous abortion (no or yes), child sex (female or male), gestational

age (in weeks), and small for gestational age according to weight (no or yes) were obtained from maternal and infant clinical records. Other variables collected at the time of neuropsychological testing were cohabitation (both parents or others), nursery school (no or yes), and mother employed when child was aged 1 year (yes or no).

Statistical Analysis

Univariate linear regression models were built to assess the relationship between MS and PS scores and mother and infant covariates. A core model was built for both the MS and the PS using all the significant covariates in the univariate analysis ($P < .20$). Following a backward elimination procedure, all the covariates associated with the MS and PS were included at a level of $P < .10$. Regardless of their statistical significance, the previous variables were held as adjustment variables in the models if they changed the magnitude of the main effects by more than 10%.

Multiple linear and logistic regression models were used to estimate associations of the MS and PS scores with usual dietary folate intake and FA supplement use.

To explore heterogeneity among cohorts, a meta-analysis was performed to obtain combined estimates of the association of MS and PS scores with usual dietary folate intake and FA supplement use. Heterogeneity was quantified with the I^2 measure³⁶ under the fixed-effects hypothesis; when heterogeneity was detected ($I^2 > 50\%$), the random-effects model was applied.

Finally, a sensitivity analysis was conducted to evaluate the robustness of the findings after exclusion of the following: preterm deliveries (92 cases), children with pathological findings that could affect infant neurodevelopment (18 cases; including hypotonia, plagiocephalia, or shortening of the sternocleidomastoid muscle), children small for gestational age (250 cases), or children with low quality of the test results because of special conditions at the time of the BSID examination (144 cases; eg, rejecting behaviors, irritability, tiredness, sleepiness, or feverish). Because some medical conditions may lead doctors to advise women to use an FA dosage higher than 5000 $\mu\text{g}/\text{d}$, we also performed a sensitivity analysis after excluding women with diabetes mellitus (6 cases) and epilepsy (9 cases). No women had a family history of neural tube defects in our study. Similarly, we carried out a sensitivity analysis after excluding other variables potentially related to the metabolism of FA such as thyroid disease (120 cases), a previous death at birth (21 cases), and body mass index (calculated as weight in kilograms divided by height in meters squared) of 40 or higher (14 cases). In total, 170 women were excluded from this sensitivity analysis. Iodine intake from supplements or fish consumption, which could be related to infant neurodevelopment, was also explored in sensitivity analyses. In addition, we evaluated the effect of the covariates small for gestational age and gestational age as potential mediators of the associations of interest. We also explored whether associations varied significantly according to child's sex because the neuropsychological development at such early ages may be different.

Table 1. Mother and Child Characteristics

Characteristic	No. (%)					P Value ^a
	Total (N = 2213)	Asturias (n = 412)	Gipuzkoa (n = 548)	Sabadell (n = 559)	Valencia (n = 694)	
Mother's age, mean (SD), y	30.8 (4.2)	31.8 (4.2)	31.4 (3.6)	30.4 (4.3)	30.1 (4.4)	<.001
Country of origin Spain	1929 (87.2)	386 (93.7)	515 (94.0)	468 (83.7)	560 (80.7)	<.001
University-level education	789 (35.6)	162 (39.3)	279 (50.9)	173 (30.9)	175 (25.2)	<.001
Social class I/II, high	495 (22.4)	93 (22.6)	169 (30.8)	117 (20.9)	116 (16.7)	<.001
Employed ^b	1383 (62.5)	253 (61.4)	383 (69.9)	380 (68.0)	367 (52.9)	<.001
Nursery school ^b	751 (33.9)	194 (47.1)	246 (44.9)	167 (29.9)	144 (20.7)	<.001
Smoking during pregnancy	637 (28.8)	92 (22.3)	122 (22.3)	152 (27.2)	271 (39.0)	<.001
Planned pregnancy	1870 (84.5)	364 (88.3)	499 (91.1)	477 (85.3)	530 (76.4)	<.001
Parity ≥1	948 (42.8)	158 (38.3)	237 (43.2)	241 (43.1)	312 (45.0)	.19
Previous abortion	510 (23.0)	93 (22.6)	113 (20.6)	142 (25.4)	162 (23.3)	.29
Child's sex female	1079 (48.8)	197 (47.8)	280 (51.1)	273 (48.8)	329 (47.4)	.54
GA, mean (SD), wk	39.6 (1.4)	39.5 (1.4)	39.7 (1.4)	39.7 (1.4)	39.6 (1.5)	.04
Small for GA ^c	215 (9.7)	29 (7.0)	44 (8.0)	64 (11.4)	78 (11.2)	.04
FA supplement dosage, µg/d						
<400	1268 (57.3)	145 (35.2)	322 (58.7)	409 (73.2)	392 (56.5)	<.001
400-1000	387 (17.5)	109 (26.5)	63 (11.5)	65 (11.6)	150 (21.6)	
1000-5000	481 (21.7)	129 (31.3)	148 (27.0)	79 (14.1)	125 (18.0)	
>5000	77 (3.5)	29 (7.0)	15 (2.7)	6 (1.1)	27 (4.0)	
Tertile of dietary folate intake, µg/d						
120-271	738 (33.3)	108 (26.2)	75 (13.7)	215 (38.5)	340 (49.0)	<.001
271-326	738 (33.3)	140 (34.0)	196 (35.8)	204 (36.5)	198 (28.5)	
326-684	737 (33.3)	164 (39.8)	277 (50.5)	140 (25.0)	156 (22.5)	
MS score, mean (SD)	98.2 (16.3)	97.9 (16.1)	98.5 (15.7)	98.5 (16.0)	97.9 (17.0)	.85
PS score, mean (SD)	99.0 (15.7)	98.9 (16.5)	98.9 (15.6)	99.3 (15.2)	98.8 (15.7)	.92
Child's age at BSID examination, median (IQR), mo	14.3 (12.6-15.4)	19.1 (18.5-20.2)	14.4 (14.0-14.9)	14.5 (14.2-14.9)	12.2 (12.0-12.5)	<.001

Abbreviations: BSID, Bayley Scales of Infant Development; FA, folic acid; GA, gestational age; IQR, interquartile range; MS, mental scale; PS, psychomotor scale.

^a From the χ^2 test (categorical variables), analysis of variance (continuous

variables), and Kruskal-Wallis test (months at examination).

^b Variables collected at the time of the neuropsychological examination.

^c According to weight.

Statistical analyses were conducted with R statistical software version 3.0.0 (R Foundation for Statistical Computing).

Results

Characteristics of the pregnant women and the children are presented in Table 1. The mean (SD) age of the mothers during pregnancy (N = 2213) was 30.8 (4.2) years, with 62.3% of the sample aged 30 years or older. Among the women, 87.2% were born in Spain; most mothers who were not born in Spain were grouped in the Mediterranean areas (Sabadell and Valencia). Concerning educational level, 39.3% and 50.9% of women had university studies in Asturias and Gipuzkoa, respectively, while only 30.9% and 25.2% had university studies in Sabadell and Valencia, respectively. In general, 62.5% of the mothers were working at the time of the neuropsychological examination. Most of the mothers planned their pregnancy (84.5%), and 57.2% of the women were nulliparous.

The distribution of usual daily folate intake and FA supplement use differed among the 4 geographic areas (both $P < .001$).

Considering the entire pregnancy, more than half the women did not reach the minimum recommended FA supplement dosages of 400 µg/d (57.3%) and 25.2% of women took more than 1000 µg/d, including 3.5% who consumed more than 5000 µg/d. The percentages of women taking FA supplements at a dosage higher than 5000 µg/d were 7.0% in Asturias and lower in the other cohorts (4.0% in Valencia, 2.7% Gipuzkoa, and 1.1% in Sabadell).

Mean daily folate intake differed by geographic area ($P < .001$). Gipuzkoa was the area with the highest mean (SD) daily folate intake (331.7 [56.2] µg/d), followed by Asturias (314.2 [71.4] µg/d), Sabadell (291.3 [59.8] µg/d), and Valencia (280.0 [61.9] µg/d). The mean total FA intake for women classified as taking an FA supplement dosage of 400 to 1000 µg/d was 862.2 µg/d (300.6 µg from diet plus 561.6 µg from supplements), and those for women taking FA supplement dosages less than 400 µg/d, 1000 to 5000 µg/d, and more than 5000 µg/d were 468.8 µg/d (300.1 µg from diet plus 168.7 µg from supplements), 2900.2 µg/d (306.9 µg from diet plus 2593.3 µg from supplements), and 5920.0 µg/d (311.1 µg from diet plus 5608.9 µg from supplements), respectively. Neuropsychological examination was performed differently among geographic regions. Chil-

Table 2. Comparison of FA Intake Variables With Infant Neurodevelopment by Geographic Area

Variable	MS Score					PS Score				
	Mean (SD)				P Value	Mean (SD)				P Value
	Asturias (n = 412)	Gipuzkoa (n = 548)	Sabadell (n = 559)	Valencia (n = 694)		Asturias (n = 412)	Gipuzkoa (n = 548)	Sabadell (n = 559)	Valencia (n = 694)	
FA supplement dosage, µg/d										
<400	99.7 (17.1)	97.6 (15.2)	99.6 (15.8)	98.5 (17.0)	.36	97.6 (16.5)	98.9 (15.6)	100.1 (14.9)	99.1 (16.1)	.38
400-1000	97.8 (17.2)	96.7 (16.0)	92.7 (16.0)	96.7 (17.0)	.27	98.6 (16.2)	99.8 (15.6)	96.5 (14.3)	100.0 (15.1)	.46
1000-5000	95.9 (14.9)	100.8 (16.3)	98.3 (15.8)	98.2 (16.6)	.08	100.3 (17.5)	99.0 (15.7)	98.4 (17.3)	97.6 (15.3)	.63
>5000	99.0 (11.3)	102.4 (15.9)	92.7 (22.7)	95.1 (19.2)	.45	100.4 (12.0)	93.6 (14.6)	91.1 (14.9)	91.7 (14.1)	.08
Tertile of dietary folate intake, µg/d										
120-271	94.6 (15.7)	95.1 (14.2)	100.0 (16.7)	98.0 (16.8)	.02	99.1 (17.5)	96.0 (15.8)	100.9 (15.9)	99.0 (14.9)	.13
271-326	100.3 (15.2)	98.4 (16.4)	96.7 (15.2)	98.2 (16.8)	.22	100.8 (16.3)	98.7 (15.2)	98.2 (15.3)	98.5 (15.6)	.46
326-684	98.1 (16.9)	99.6 (15.5)	99.0 (16.0)	97.4 (17.7)	.54	97.2 (15.8)	99.8 (15.8)	98.6 (13.8)	98.5 (17.6)	.41

Abbreviations: FA, folic acid; MS, mental scale; PS, psychomotor scale.

Table 3. Association Between FA Intake Variables During Pregnancy and Infant MS and PS Scores^a

Variable	MS Score				PS Score			
	Continuous		MS Score <85		Continuous		PS Score <85	
	β (95% CI)	I ² , % ^b	OR (95% CI)	I ² , % ^b	β (95% CI)	I ² , % ^b	OR (95% CI)	I ² , % ^b
FA supplement dosage, µg/d								
<400	2.30 (0.38 to 4.22)	33.3	0.51 (0.31 to 0.84)	64.5 ^c	0.02 (-1.86 to 1.89)	0.0	1.17 (0.85 to 1.62)	0.0
400-1000	1 [Reference]		1 [Reference]		1 [Reference]		1 [Reference]	
1000-5000	1.53 (-0.68 to 3.75)	48.8	0.62 (0.44 to 0.86)	16.5	-0.42 (-2.62 to 1.76)	0.0	1.30 (0.89 to 1.88)	0.0
>5000	0.57 (-3.43 to 4.58)	0.0	0.62 (0.32 to 1.20)	0.0	-4.35 (-8.34 to -0.36)	14.4	1.59 (0.82 to 3.08)	29.6
Tertile of dietary folate intake, µg/d								
120-271	1 [Reference]		1 [Reference]		1 [Reference]		1 [Reference]	
271-326	1.86 (-1.40 to 5.12)	69.8 ^c	0.78 (0.47 to 1.29)	67.4 ^c	-0.01 (-1.72 to 1.70)	38.1	0.93 (0.55 to 1.56)	67.1 ^c
326-684	2.02 (0.19 to 3.86)	33.4	0.88 (0.66 to 1.17)	0.0	-0.18 (-1.99 to 1.62)	28.9	1.03 (0.77 to 1.39)	36.5

Abbreviations: FA, folic acid; MS, mental scale; OR, odds ratio; PS, psychomotor scale.

^a Cohort-specific models were combined using meta-analysis. All models for infant neuropsychological scales were adjusted by maternal age (in years), country of origin (Spain or other), social class (I/II [high], III, or IV/V [low]), employed when the child was aged 1 year (yes or no), and child's sex (female or male).

^b We used the results from the fixed-effects meta-analysis model when I² was less than 50% and from the random-effects meta-analysis model when I² was greater than 50%.

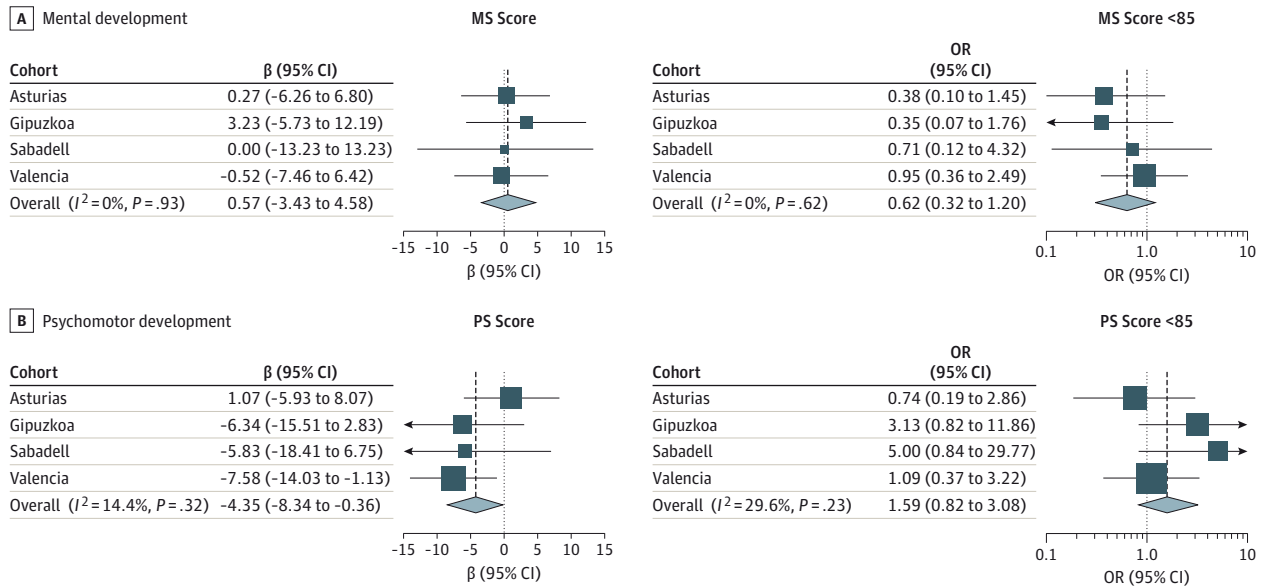
^c P_{Cochran Q} < .05 from test of heterogeneity.

dren were evaluated earlier in Valencia (median age, 12.2 months) than in the other areas, at almost the same time in Gipuzkoa and Sabadell (median ages, 14.4 and 14.5 months, respectively), and later in Asturias (median age, 19.1 months). There were no statistically significant differences between areas for MS scores ($P = .85$) or PS scores ($P = .92$).

Table 2 presents the MS and PS scores by geographic area according to use of FA supplements and usual dietary folate intake. For FA supplement intakes higher than 5000 µg/d, mean PS scores were lower in Gipuzkoa (93.6), Sabadell (91.1), and Valencia (91.7) and, to a lesser extent, mean MS scores were lower in Sabadell (92.7) and Valencia (95.1). However, differences in PS scores were not statistically significant ($P = .08$). Statistically significantly lower MS scores ($P = .02$) were observed in Asturias and Gipuzkoa among those in the lowest tertile of dietary folate intake compared with the other geographic areas.

In multiple regression analysis for all cohorts combined (Table 3), after adjusting for dietary intake of FA and other covariates, we found a significantly higher mean MS score for infants whose mothers used FA supplements at a dosage lower than 400 µg/d ($\beta = 2.30$; 95% CI, 0.38 to 4.22) and a lower risk of delayed mental development (for an MS score <85, odds ratio = 0.51; 95% CI, 0.31 to 0.84) compared with those whose mothers were taking 400 to 1000 µg/d. A protective effect against delayed mental development was also observed for children of mothers consuming FA supplements at a dosage of 1000 to 5000 µg/d (for an MS score <85, odds ratio = 0.62; 95% CI, 0.44 to 0.86) compared with those whose mothers consumed 400 to 1000 µg/d. We also found that children whose mothers used FA supplement dosages higher than 5000 µg/d had a significantly lower mean PS score than those whose mothers consumed 400 to 1000 µg/d ($\beta = -4.35$; 95% CI, -8.34 to -0.36); however, the risk of delayed psychomotor development was

Figure 1. Pooled Estimates of the Association Between Folic Acid Supplements (400-1000 vs >5000 µg/d) and Infant Neuropsychological Development



A, Mental development. B, Psychomotor development. MS indicates mental scale; OR, odds ratio; and PS, psychomotor scale.

not statistically significant compared with those whose mothers consumed 400 to 1000 µg/d (for a PS score <85, odds ratio = 1.59; 95% CI, 0.82 to 3.08). Usual dietary folate intake was not related to MS score or PS score, except children whose mothers had intakes in the range of 326 to 684 µg/d, who had an MS score 2.02 points higher (95% CI, 0.19 to 3.86) than children of mothers with the lowest intakes (120-271 µg/d). All these results were homogeneous among cohorts ($I^2 < 50\%$) except the association between an MS score lower than 85 and an FA supplement intake lower than 400 µg/d (variation in estimate attributable to heterogeneity: $I^2 = 64.5\%$; $P_{\text{Cochran Q}} = .04$). The estimates for the usual folate intake in the range of 271 to 326 µg/d were also heterogeneous in MS score ($I^2 = 69.8\%$; $P_{\text{Cochran Q}} = .02$), MS score lower than 85 ($I^2 = 67.4\%$; $P_{\text{Cochran Q}} = .03$), and PS score lower than 85 ($I^2 = 67.1\%$; $P_{\text{Cochran Q}} = .03$). Consequently, this combined estimate was obtained from random-effects meta-analysis. No change in results was observed when the time of starting intake of FA supplements, other types of supplements (eg, iodine), or foods such as fish that could be related to neurodevelopment were taken into account (data not shown).

To examine the consistency of the findings among the geographic areas, we used forest plots and meta-analyses to pool the estimates of the association between FA supplement intake (400-1000 vs >5000 µg/d) and infant mental and psychomotor development (Figure 1). The statistically significant association observed for PS score ($\beta = -4.35$; 95% CI, -8.34 to -0.36) was based in the negative coefficients found in 3 cohorts, particularly among children in Valencia, in whom a statistically significant association was found ($\beta = -7.58$; 95% CI, -14.03 to -1.13).

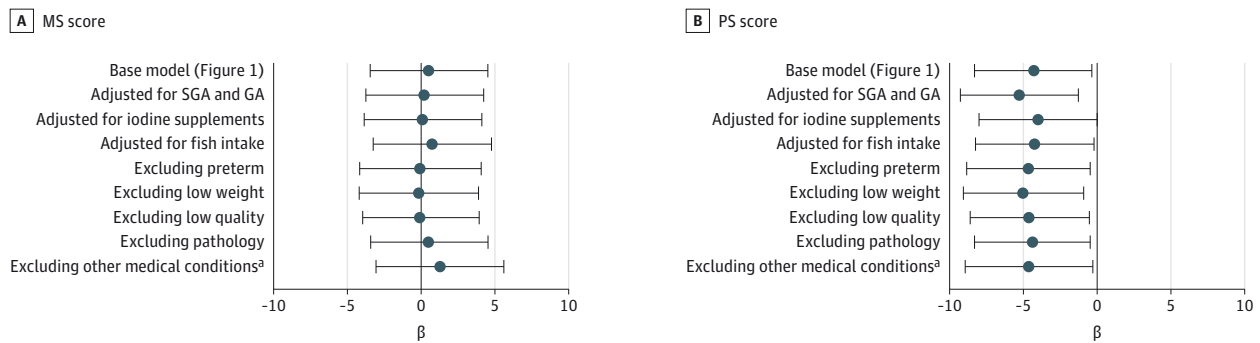
Figure 2 presents sensitivity analyses for the association between FA supplement intakes higher than 5000 µg/d and MS

and PS scores compared with FA supplement intakes of 400 to 1000 µg/d. The mean MS score ($\beta = 0.57$; 95% CI, -3.43 to 4.57) was substantially reduced when variables such as preterm children ($\beta = -0.01$; 95% CI, -4.14 to 4.13), low weight ($\beta = -0.15$; 95% CI, -4.21 to 3.91), and a low-quality BSID test ($\beta = 0.01$; 95% CI, -3.95 to 3.97) were excluded from the models, but it increased when fish intake was included in the model ($\beta = 0.80$; 95% CI, -3.22 to 4.82) and when all women with medical conditions potentially related to using an FA supplement dosage higher than 5000 µg/d were excluded from the models ($\beta = 1.31$; 95% CI, -3.03 to 5.65). The association for PS score became stronger when small for gestational age and gestational age were included in the model ($\beta = -5.30$; 95% CI, -9.32 to -1.28), when children with low weight were excluded ($\beta = -5.01$; 95% CI, -9.09 to -0.93), and, to a lesser extent, when women with medical conditions potentially related to using an FA supplement dosage higher than 5000 µg/d were excluded ($\beta = -4.64$; 95% CI, -8.96 to -0.33), although no major changes in results were observed overall. No statistically significant interaction term was found between FA supplement use or usual folate intake and child's sex (MS score: FA supplement use vs sex, $P = .23$; usual folate intake vs sex, $P = .45$; PS score: FA supplement use vs sex, $P = .46$; usual folate intake vs sex, $P = .07$).

Discussion

Our findings suggest that taking high doses of FA supplements during pregnancy is associated with lower psychomotor development of infants at 1 year of age. Compared with an FA supplement consumption of 400 to 1000 µg/d, the consumption of more than 5000 µg/d during pregnancy was as-

Figure 2. Sensitivity Analysis of the Pooled Estimates of the Association Between Folic Acid Supplements (400-1000 vs >5000 $\mu\text{g}/\text{d}$) and Infant Neuropsychological Development



A, Mental scale (MS) score. B, Psychomotor scale (PS) score. Error bars indicate 95% CI; GA, gestational age (in weeks); and SGA, small for GA (according to weight; no or yes).

³ Women with diabetes mellitus, epilepsy, body mass index (calculated as weight in kilograms divided by height in meters squared) higher than 40, thyroid disease, or a previous death at birth were excluded.

sociated with a significant decrease in PS scores in the overall sample. To our knowledge, this is the first time this association has been reported.

In contrast to our findings, some studies have reported a positive association between the consumption of FA supplements during pregnancy and child psychomotor development.^{7,8} This discrepancy may be mainly due to differences in the supplementation patterns, the duration of the supplementation period, and the follow-up of the population sample in every study. Moreover, the age range of children in these studies (3 and 4 years, respectively) and the different tests used to evaluate neuropsychological development may affect the interpretation of the study findings. The same explanation could be attributed to the lack of association with a delay of psychomotor development reported by other studies,^{13,19} although one of these studies was conducted with children at an age (18 months) similar to the age we used and with high dosages of FA supplements (5000 or >5000 $\mu\text{g}/\text{d}$). It is significant that few studies have measured total dietary folate intake and FA supplements at different stages of pregnancy as we did. We explored the association of using a dosage higher than 5000 $\mu\text{g}/\text{d}$ in the first or second period of pregnancy on PS score. We still observed a lower negative association in both periods, although the associations were not statistically significant ($\beta = -1.64$ and -1.99 , respectively), with some evidence of heterogeneity for the second period (data not shown). Nevertheless, the main significant association found in our study ($\beta = -4.35$) was based on women using high dosages during the entire pregnancy.

The current recommendation for FA supplements is based on solid evidence that FA reduces the incidence of neural tube defects³⁷ and other birth outcomes,¹ although the consequences of long-term use of FA supplements remain unclear. Thus, further studies are required to determine whether the use of very high dosages of FA supplements during pregnancy may have adverse consequences on infant neurodevelopment, particularly given the current growth in FA supplement consumption observed in several pregnant populations.³⁸⁻⁴⁰ In contrast to natural dietary folate, FA is a synthetic oxidized form

that must be metabolized to coenzymatic tetrahydrofolate derivatives⁴¹ to be biologically active. In recent studies, the concern about the presence of unmetabolized FA has been increasing because of the intake of high dosages, especially in women who take supplements during pregnancy.^{41,42} However, there is still no clear evidence about the association with adverse health effects, although some animal studies have recently indicated the possibility of detrimental effects on child development because of high FA intake.^{25,26}

A previously reported study in the Valencia cohort²⁴ found that high dosages of FA supplements were significantly associated with reduced fetal growth. Some evidence regarding the link between fetal growth and infant psychomotor development is already available.^{43,44} Thus, fetal growth may be considered a good predictive factor of infant psychomotor development, although there may be other relevant factors. When children who were small for gestational age were excluded from the analysis, the negative association between high FA dosages and PS score was strengthened, which may suggest that factors other than lower fetal growth (eg, high FA supplement dosages) may have some role on infant psychomotor development.

In our study, the overall estimates indicated that usual dietary folate intake and FA supplements during pregnancy were associated with improved MS scores. These findings are in accordance with those observed in infants at similar ages¹³ and in older children.^{8,14,16} In contrast, the lack of association reported by other studies^{18,19} can be explained by the differences in the time and/or dosage of measuring exposure and in the characteristics of the population, although the children's age and the different tests used to evaluate neuropsychological development should also be considered. We have no explanation for the unexpected result of the higher mean MS scores on the BSID or the protective effect against delayed mental development for children of mothers who took less than the recommended FA dosage (<400 $\mu\text{g}/\text{d}$) or more than the recommended dosage (1000-5000 $\mu\text{g}/\text{d}$) in comparison with women taking the recommended dosage. It should be considered that women in the lowest category had an average daily FA intake of 468.8 μg and those in the reference category had an average

daily FA intake of 862.2 μg , and heterogeneity among geographic areas could also play a role (estimates were based on random-effects models). We must also consider that some estimates were based on low numbers, a limitation of our study. Nevertheless, the inverse effect on early motor development that we found may affect later mental development. Several studies⁴⁵⁻⁴⁷ have highlighted the strength of this relationship, providing evidence that early motor development is an essential agent for future developmental patterns. Consequently, our findings can be extended by further multiple measures of neuropsychological functions repeated throughout childhood and adolescence to provide more firm conclusions and to support the need for ongoing research into potential etiological factors of disturbances of development over time.

This study has several limitations. We adjusted for a wide range of potential risk factors, although the effects of other potential confounding factors or modifiers cannot be discarded. Regarding FA supplement use, it can be argued that mothers with higher-risk pregnancy are more likely to be encouraged to take supplements. We did not collect information on the reasons for mothers taking an FA supplement dosage higher than 5000 $\mu\text{g}/\text{d}$, although when we excluded women with diabetes, taking anticonvulsive medications, or with a family history of neural tube defects, estimates for PS score remained unchanged and statistically significant. Similarly, when we excluded women with other conditions potentially related to the metabolism of FA such as thyroid disease, a previous death at birth, or a body mass index of 40 or higher, results remained very similar. However, use of multivitamins is part of routine supplementation programs implemented during pregnancy, and these programs were started at the middle or the end of the recruitment period. We observed no association of usual dietary folate intake and FA supplement use with maternal characteristics except maternal age, country of origin, social class, and being employed when the child was aged 1 year, but we accounted for these variables in the multivariate models. Another limitation may be that we did not account for maternal IQ and mental health, although we did adjust for sociodemographic covariates that may partly correct for them.

Unfortunately, it was not possible to replicate our findings against serum levels of FA, although nutrient intake es-

timates from diet and supplements by FFQ obtained good validity when compared with FA concentration in blood in a group of women, thus reducing a possible bias through incorrect classification of exposure.³² Although some misclassification of diet is possible, any inaccuracy in reporting diet by women should not be differential, thereby reinforcing the association found.

Nevertheless, our study has several strengths. The multicenter structure of this study and the wide range of usual dietary folate intake and FA supplement patterns provide results that can be extrapolated to a wide range of situations. The consistency of the results related to continuous outcomes by cohort and the sensitivity analyses applied reinforce these findings. Moreover, the prospective cohort design of the INMA Project study allows for the control of a wide variety of confounding variables and for the blinded and homogeneous conditions in which the children of women using and not using FA supplements were evaluated. We are aware of the limited validity of the BSID at this early age for predicting later cognitive and psychomotor performance, but the INMA Project study promotes the opportunity to verify long-term effects in future assessments throughout childhood and adolescence to identify potential etiological factors of disturbances of normal development over time.

Conclusions

This study shows the potential detrimental effect of high dosages of FA supplements during pregnancy on infant psychomotor development. Our findings add to other evidence supporting a possible negative effect of the use of FA supplement dosages higher than recommended.²³⁻²⁶ The relevance of our findings is highlighted by the incipient evidence regarding the inappropriate use of FA supplements in Spanish pregnant women.³⁸⁻⁴⁰ The benefits of standard FA dosage recommendations are well established and should be maintained for the periconceptual period. However, compliance with FA supplementation policies should be monitored to avoid the use of an FA supplement dosage higher than 5000 $\mu\text{g}/\text{d}$ during pregnancy unless medically indicated and until the safety of such a high dosage is confirmed in further studies.

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