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Folic Acid Use in Pregnancy and the Development of Atopy, Asthma, and Lung Function in Childhood

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KEY WORDS

asthma, cohort studies, child, folic acid, pregnancy

ABBREVIATIONS

AD—atopic dermatitis

IgE—immunoglobulin E

ICF—intracellular folic acid

ISAAC—International Study of Asthma and Allergies in Childhood

FEV₁—forced expiratory volume in 1 second

%FEV₁—percentage of predicted forced expiratory volume in 1 second

FVC—forced vital capacity

%FVC—percentage of predicted forced vital capacity

GEE—generalized estimating equation

OR—odds ratio

CI—confidence interval

aOR—adjusted odds ratio

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WHAT'S KNOWN ON THIS SUBJECT: Folic acid use during pregnancy has been promoted worldwide for the prevention of neural tube defects. Recently, folic acid supplementation during pregnancy has been implicated as a potential risk factor for the development of atopic diseases in early childhood.



WHAT THIS STUDY ADDS: This study prospectively investigated the relationship between maternal folic acid use during pregnancy and the development of atopic manifestations, using questionnaire information as well as measured intracellular folic acid levels in late pregnancy. The latter has not been investigated previously.

abstract

BACKGROUND: Recently, folic acid supplementation during pregnancy was implicated as a potential risk factor for atopic diseases in childhood.

OBJECTIVE: To investigate whether folic acid supplementation and higher intracellular folic acid (ICF) levels during pregnancy increase the risk of childhood atopic diseases.

METHODS: In the KOALA Birth Cohort Study ($N = 2834$), data on eczema and wheeze were collected by using repeated questionnaires at 3, 7, 12, and 24 months, 4 to 5 years, and 6 to 7 years after delivery. Atopic dermatitis and total and specific immunoglobulin E levels were determined at age 2 years and asthma and lung function at age 6 to 7 years. We defined folic acid use as stand-alone and/or multivitamin supplements according to the period of use before and/or during pregnancy. ICF levels were determined in blood samples taken at ~35 weeks of pregnancy ($n = 837$). Multivariable logistic and linear regression analyses were conducted, with generalized estimating equation models for repeated outcomes.

RESULTS: Maternal folic acid supplement use during pregnancy was not associated with increased risk of wheeze, lung function, asthma, or related atopic outcomes in the offspring. Maternal ICF level in late pregnancy was inversely associated with asthma risk at age 6 to 7 years in a dose-dependent manner (P for trend = .05).

CONCLUSIONS: Our results do not confirm any meaningful association between folic acid supplement use during pregnancy and atopic diseases in the offspring. Higher ICF levels in pregnancy tended, at most, toward a small decreased risk for developing asthma. *Pediatrics* 2011; 128:e135–e144

For many years, folic acid supplementation before and during the first trimester of pregnancy has been promoted worldwide.^{1,2} A 23% to 78% reduction of neural tube defects has been shown if 200 to 400 μg of folic acid as a supplement is taken daily during pregnancy.^{3,4} In addition, the risk of other congenital malformations can be reduced with an adequate folic acid status in pregnancy.^{4–7}

Contrary to well-studied positive effects, little research has been conducted on the possible adverse effects of folic acid supplementation during pregnancy.⁸ Folic acid functions as a methyl donor in biochemical reactions,^{1,2,9,10} and it has been hypothesized that through DNA-methylation, a methyl donor-rich diet could increase the risk of developing allergic diseases, as has been seen in mice.^{11–13}

To our knowledge, only 2 studies have investigated the relationship between folic acid supplementation during pregnancy and childhood airway disease. Håberg et al¹ demonstrated that folic acid supplement use early in pregnancy was associated with a small increased risk for wheezing in the offspring during the first 18 months of life. Recently, Whitrow et al¹⁴ reported that folic acid supplementation in late pregnancy was associated with an increased risk of childhood asthma. None of the studies in children included measurements of maternal folic acid levels in pregnancy, but a recent study among adults found that folic acid deficiency was associated with physician-diagnosed asthma.¹⁵ However, Granell et al¹⁶ and Thuesen et al¹⁷ found no evidence of an association between impaired folate metabolism and the development of atopy in children or adults. Most recently, Håberg et al,¹⁸ in an observational study, demonstrated that high maternal plasma folate levels (>17.84 nmol/L) in the second trimester of pregnancy was associated with an in-

creased risk of asthma in the offspring at 3 years of age.

The present study aims to prospectively investigate the relationship between maternal folic acid use during pregnancy and the development of atopic manifestations, including eczema, atopic dermatitis (AD), total immunoglobulin E (IgE), allergic sensitization (specific IgE), wheeze, and asthma and lung function until age 6 to 7 years, using questionnaire information on supplement use as well as measured intracellular folic acid (ICF) levels.

METHODS

Study Population

The present study was conducted within the prospective KOALA Birth Cohort Study (in Dutch, the Child, Parent and Health: Lifestyle and, Genetic Constitution study) in the Netherlands with the goal of investigating early life risk factors for atopy and asthma. The study design has been previously described in detail.¹⁹ In summary, from October 2000, healthy pregnant women were recruited in week 10 to 14 of their pregnancy from an ongoing prospective cohort study on pregnancy-related pelvic girdle pain ($N = 7020$)²⁰; these individuals comprised the conventional recruitment group ($n = 2343$). In addition, pregnant women were recruited through posters in organic food shops, anthroposophical physician offices, and midwives at 10 to 14 weeks of pregnancy; these individuals comprised the alternative recruitment group ($n = 491$). Information on supplement use during pregnancy and atopic outcomes in the offspring were all prospectively collected by using repeated questionnaires during pregnancy (weeks 14 and 34) and at ages 3, 7, 12, and 24 months, 4 to 5 years, and 6 to 7 years. Venous blood samples were taken at ~ 35 weeks of pregnancy to determine ICF levels ($n = 837$). Children of women

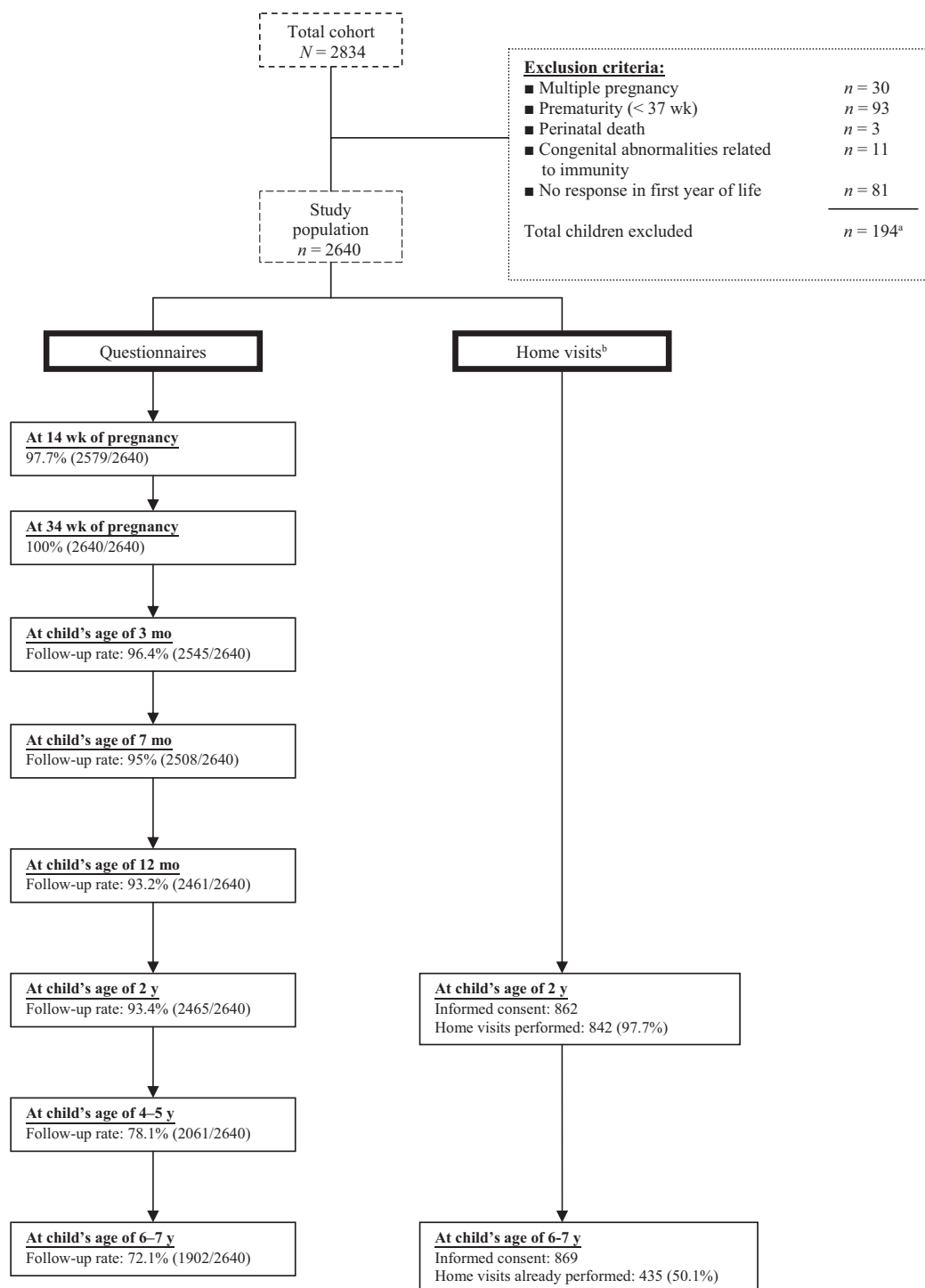
who had provided blood samples in pregnancy were candidates for the home visits. During the home visit, at age 2 years ($n = 842$), manifestations of AD were assessed and a blood sample was taken for determining total and specific IgE levels. Currently, home visits are ongoing to assess lung function (Fig 1).

Informed consent forms were signed by all parents. Ethical approval for the KOALA study was obtained from the medical ethics committee of the Maastricht University/University Hospital of Maastricht and the Netherlands Central Committee for Medical Studies on Human Subjects.

Exclusion criteria were: multiple pregnancies, birth at <37 weeks of gestation, perinatal death, congenital abnormalities related to immunity (Down syndrome), and no response to any of the questionnaires in the first year of life. Ultimately, 2640 children were included in the study.

Folic Acid Use

To assess folic acid supplement use during pregnancy, the following question was included in the questionnaire completed in weeks 14 and 34 of pregnancy: “Did you take folic acid, either as a stand-alone supplement or as part of a multivitamin supplement, before or during your pregnancy?” Women were also asked when they had started taking supplements: before or after conception and in which trimester (first, second, and/or third). We categorized subjects into 2 categories: any use of folic acid versus no use. Additionally, we divided the folic acid users into early users (use during the advice period [4 weeks before until 8 weeks after conception] and/or use solely during the first trimester), use during whole pregnancy, and a “rest” category (users that did not fit in any of these categories [eg, use solely during

**FIGURE 1**

Flowchart for inclusion in the study cohort and follow-up. ^a Some belong to 2 groups. Biosampling and home visits were performed in participants recruited from January 2002 onward. Therefore, not all children were offered a home visit.

the second and third trimesters]). To eliminate the possibility that other vitamins in the multivitamin supplements could be the cause of a possible association, we also analyzed folic acid use as a stand-alone supplement.

ICF Levels

In the blood samples, taken in week 35 of pregnancy, fluorimmunoassay was used to measure lysed folic acid in erythrocytes. The concentration of folic acid in erythrocytes was calcu-

lated by using the following formula: lysed folic acid (nmol/L) $\times$ 23 (dilution factor) $\times$ (100/hematocrit %). For the statistical analyses, quintiles were determined on the basis of the distribution in the total cohort.

Eczema

Eczema was assessed at age 7, 12, and 24 months and 6 to 7 years with the following International Study of Asthma and Allergies in Childhood (ISAAC)²¹ question: "Did your child suffer from an itchy rash that was coming and going in the past months?" If this question was answered affirmatively, infants were classified as having developed eczema. Children were classified as having no eczema if the parents did not report eczema in any of the 4 questionnaires, or if only diaper rash, rash around the eyes, and/or scalp scaling was reported.

Atopic Dermatitis

A trained nurse examined the child for manifestations of AD during the home visit at the child's age of 2 years using the UK Working Party criteria, described earlier.²² In summary, the probability of AD was derived from the presence of 4 clinical symptoms: (1) itchy rash; (2) history of flexural dermatitis; (3) visible flexural dermatitis; and (4) onset before the age of 2 years. Children with a UK Working Party probability of >0.9 were considered children with AD, as previously described in detail.²³

Allergic Sensitization and Total IgE Levels

Total and specific IgE levels were determined in 815 venous blood samples that were taken at age 2 years. Total serum IgE levels were measured by using radioimmunoassay, with a detection limit of <0.50 IU/mL, as described earlier.^{24,25} Increased total IgE level was defined as >25.0 IU/mL, based on the 67th percentile of the distribution in the total cohort.

A radioallergosorbent test^{24,25} with a detection limit of <0.13 IU/mL was used to determine the serum level of specific IgE against hen's egg, cow's milk, peanut, birch pollen, grass pol-

len, cat dander, dog dander, and house dust mite. Allergic sensitization was defined as a specific IgE level >0.3 IU/mL for ≥ 1 of the tested allergens.

Wheeze

Wheeze was assessed by the following ISAAC question²¹ in the questionnaires at age 2 years, 4 to 5 years, and 6 to 7 years: "Did your child suffer from wheezing in the last 12 months?" Using the same ISAAC question, at child's age 3, 7, and 12 months, parents were asked if their child suffered from wheezing since the last follow-up.

Asthma

Asthma was defined as ever physician-diagnosed asthma with clinical symptoms and/or the use of asthma medication in the last 12 months. Clinical symptoms were defined as having had at least 1 attack of wheeze or dyspnea in the last 12 months. The use of asthma medication was defined as regular use (everyday use during at least 2 months or use associated with physical activity) of short-acting inhalation bronchodilators or the use of inhaled corticosteroids and medication use according to the Dutch guidelines of treatment of bronchial asthma in children.²⁶

Assessment of asthma symptoms was based on the following ISAAC questions at age 6 to 7 years: "Did your child suffer from wheezing in the last 12 months?" and "Did your child suffer from shortness of breath in the last 12 months?" We assessed physician's diagnosis and asthma medication use with the questions: "Did a physician ever diagnose asthma in your child?" and "Did your child use medication for asthma or wheezing in the last 12 months, and if so, what medication?"

Lung Function

During the home visit at the child's age of 6 to 7 years, spirometry was per-

formed by a trained research assistant using the handheld Medikro SpiroStar USB spirometer (Medikro Oy, Kuopio, Finland). Forced expiratory volume in 1 second (FEV₁) and forced vital capacity (FVC) were assessed as percentage of the predicted FEV₁ (%FEV₁) and predicted FVC (%FVC), with gender, age, ethnicity, weight and height, ambient temperature, humidity, and barometric pressure taken into account. The testing protocol followed the guidelines of the American Thoracic Society and the European Respiratory Society.²⁷ Testing was performed when the child was sitting and continued until at least 3 acceptable curves were achieved, with a maximum of 8 attempts.

Statistical Analysis

Data were analyzed by using SPSS 15.0 for Windows (SPSS Inc, Chicago, IL). Univariable and multivariable logistic regression was used for the association between folic acid use and ICF levels during pregnancy and asthma, allergic sensitization, total IgE, and AD. Linear regression was used for lung function outcomes. Generalized estimating equation (GEE) models with an exchangeable correlation structure were used for repeated outcomes: wheeze and eczema.

All analyses were adjusted for potential confounders: recruitment group, maternal antibiotic, smoking and alcohol use during pregnancy, mode and place of delivery, birth weight, gender, treatment with antibiotics during the first 6 months of life, exposure to environmental tobacco smoke and domestic animals, breastfeeding, maternal education level, family history of atopy, siblings, day care attendance, and multivitamin or other supplement use during pregnancy. We adjusted for the latter to exclude the possibility that other vitamins included in multivitamin or other supplements interfered with the

association investigated in the present study.

In the GEE analysis, we adjusted for exposure to environmental tobacco smoke at each moment of measurement separately (3, 7, 12, and 24 months, 4–5 years, and 6–7 years).

Participants were recruited through 2 different recruitment channels; we therefore tested for interactions between folic acid exposure and recruitment group. Because the interaction terms did not reach statistical significance for any of the outcomes ($P < .05$), participants from both recruitment sources were pooled for the analyses. Likewise, no statistically significant interaction was found between folic acid exposure and family history of atopy.

RESULTS

Table 1 shows that the distribution of population characteristics is fairly similar over the categories of folic acid use. In the conventional recruitment group, folic acid is used more during pregnancy than in the alternative recruitment group; findings were similar for families in whom both parents are atopic. Women that did not use folic acid were less likely to have given antibiotics to their child.

No major differences were observed in the distribution of characteristics of the cohort at birth, at 2 years, and after 6 to 7 years of follow-up (Supplemental Table 4). Also, in the group of children with lung function measurements, the distribution of characteristics was very similar to that of the total cohort (data not shown).

The prevalence of wheeze and eczema from birth to age 6 to 7 years is shown in Fig 2. A total of 130 children (6.8%) were classified as having developed asthma at 6 to 7 years of age. At age 2 years, allergic sensitization was present in 220 of 799 children (27.5%); 269 of 807 children (33.3%) had elevated

levels of total serum IgE; and 113 of 842 children (13.4%) had AD.

Folic acid use during pregnancy was not associated with increased risk of developing eczema, AD, high total IgE level, allergic sensitization, wheeze, or asthma in the child (Table 2). Eczema risk in the child was increased when the mother had used folic acid during whole pregnancy (odds ratio [OR]: 1.34 [95% confidence interval (CI): 1.02–1.75]) but lost statistical significance after adjusting for confounders (OR: 1.27 [95% CI: 0.96–1.66]). High serum total IgE level at 2 years was inversely associated with folic acid use at some point during pregnancy (OR: 0.68 [95% CI: 0.46–0.99]), but this was not statistically significant after adjustment for confounders (OR: 0.71 [95% CI: 0.47–1.07]).

We found no statistically significant association for folic acid use as a stand-alone supplement versus nonusers or for early users (use at least during the first trimester) versus nonusers (data not shown).

ICF level in the third trimester of pregnancy was not associated with most atopic outcomes. A decreased risk for allergic sensitization was seen in the second ICF quintile (OR: 0.47 [95% CI: 0.23–0.99]) compared with the first (reference) quintile, but this was not seen in higher quintiles (Table 3). Furthermore, ICF level was associated with a decreased asthma risk at age 6 to 7 years in a dose-dependent manner (P for trend = 0.05).

The mean %FVC was 95.41 (SD: 11.60), and the mean %FEV₁ was 101.13 (SD: 12.86). No statistically significant association was found between folic acid use and ICF levels during pregnancy and the child's %FVC or %FEV₁ (Tables 2 and 3).

DISCUSSION

In the present study, we found no significant association between folic acid supplement use during pregnancy and

atopic outcomes and lung function in the offspring. Higher maternal ICF levels in late pregnancy were associated with decreased asthma risk at age 6 to 7 years in a dose-dependent manner. Folic acid levels strongly correlate with folic acid supplement use ($P < .01$).^{28,29} However, because of the small numbers in our study, folic acid supplement use solely in late pregnancy could not be analyzed separately.

We hypothesized that folic acid supplementation, specifically during early pregnancy, promotes the development of atopic diseases in the offspring. Folic acid functions as a methyl donor,^{1,2,9,10} and several studies revealed that the expression of certain genes (eg, *FoxP3*, *STAT4*) and regulatory T-cell differentiation is enhanced by hypomethylation of naive T cells.^{11,13} This factor can lead to the expansion of T-helper 1 cells and thus to a T-helper 1 phenotype, suggesting that hypomethylation could prevent allergic airway disease.^{11,13} Indeed, in a mouse model, in utero exposure to a methyl donor-rich diet increased airway hyperreactivity, as well as total and specific IgE levels, and induced histologic changes consistent with allergic airway disease.¹¹ This finding was not seen for exposure solely during lactation or adulthood,^{8,11} indicating different effects on asthma and atopy development at various stages in life, as was also suggested for the different stages in pregnancy.¹⁴

Similar to our results, Whitrow et al¹⁴ found no increased risk for asthma with folic acid use in early pregnancy. In contrast, Håberg et al¹ demonstrated that use of folic acid during early pregnancy was associated with a higher risk of wheezing early in life. However, the observed effect was small (OR: 1.06 [95% CI: 1.03–1.10]). Although our study was not designed to be able to detect ORs for wheeze weaker than 1.15 (with an α level of 5%

TABLE 1 Population Characteristics

Characteristic	Folic Acid Use During Pregnancy ^a							
	Not at All		During Early Pregnancy ^b		During Whole Pregnancy		During Another Period	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
Overall group	356	13.5	851	32.2	820	31.1	613	23.2
Recruitment group								
Conventional	251	70.5	682	80.1	733	89.4	513	83.7
Alternative	105	29.5	169	19.9	87	10.6	100	16.3
Antibiotics used by mother during pregnancy								
Yes	33	9.3	101	11.9	107	13.0	94	15.3
No	323	90.7	750	88.1	713	87.0	519	84.7
Maternal smoking during early pregnancy ^c								
Yes	27	7.6	52	6.1	52	6.4	50	8.2
No	329	92.4	799	93.9	768	93.6	563	91.8
Maternal smoking during late pregnancy ^d								
Yes	26	7.3	55	6.5	55	6.7	49	8.0
No	330	92.7	796	93.5	765	93.3	564	92.0
Maternal alcohol use during early pregnancy ^c								
Yes	40	11.2	114	13.4	64	22.0	61	10.0
No	316	88.8	737	86.6	756	78.0	552	90.0
Maternal alcohol use during late pregnancy ^d								
Yes	30	8.4	65	7.6	49	6.0	39	6.4
No	326	91.6	786	92.4	771	94.0	574	93.6
Mode and place of delivery								
Vaginal birth at home	170	47.8	377	44.3	353	43.1	280	45.7
Vaginal birth at the hospital	141	39.6	355	41.7	334	40.7	249	40.6
Cesarean delivery	29	8.1	96	11.3	102	12.4	55	9.0
Other	16	4.5	23	2.7	31	3.8	29	4.7
Birth weight								
<2500 g	3	0.8	11	1.3	18	2.2	6	1.0
2500–4500 g	343	96.4	810	95.2	784	95.6	596	97.2
>4500 g	10	2.8	30	3.5	18	2.2	11	1.8
Gender								
Male	170	47.8	422	49.6	428	52.2	314	51.2
Female	186	52.2	429	50.4	392	47.8	299	48.8
Breastfeeding								
Never	51	14.3	122	14.3	112	13.7	111	18.1
0–3 mo	88	24.7	251	29.5	260	31.7	157	25.6
4–6 mo	47	13.2	139	16.3	131	16.0	97	15.8
7–9 mo	53	14.9	132	15.5	121	14.7	81	13.2
>9 mo	85	23.9	148	17.4	122	14.9	111	18.1
Treatment with antibiotics during first 6 mo of life								
Yes	36	10.1	137	16.1	150	18.3	113	18.4
No	320	89.9	714	83.9	670	81.7	500	81.6
Exposure to domestic animals during pregnancy and the first 2 y of life								
Yes	178	50.0	447	52.5	439	53.5	339	55.3
No	142	39.9	351	41.2	312	38.0	238	38.8
Type of day care during first 2 y of life								
Parent	145	40.7	285	33.5	263	32.1	222	36.2
Guest parents	110	31.0	208	24.4	213	26.0	149	24.3
Day nursery	89	25.0	306	36.0	297	36.2	212	34.6
Combination guest parents/day nursery	12	3.3	52	6.1	47	5.7	30	4.9
Exposure to environmental tobacco smoke in first 6 to 7 y of life								
Yes	263	73.9	623	73.2	648	79.0	472	77.0
No	93	26.1	228	26.8	172	21.0	141	23.0
Siblings								
No siblings	119	33.4	342	40.2	337	41.1	229	37.4
≥1 older siblings	222	62.4	485	57.0	457	55.7	360	58.7
Atopic predisposition								
1 or both parent(s) atopic	178	50.0	465	54.6	491	60.0	362	59.1
No parent atopic	178	50.0	386	45.4	329	40.0	251	40.9
Maternal education level ^e								
Low	29	8.1	82	9.6	78	9.5	75	12.2
Middle	264	74.2	590	69.3	594	72.4	426	69.5
High	44	12.4	140	16.5	102	12.4	82	13.4
Other	13	3.6	31	3.6	35	4.3	22	3.6

Numbers may not add up to total because of missing values.

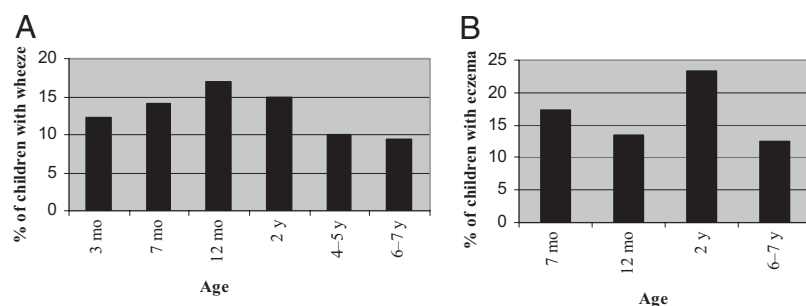
^a Folic acid use was defined as a multivitamin containing folic acid supplement or folic acid stand-alone supplement use.

^b The use during the advice period (4 weeks before conception until 8 weeks after conception) and/or use solely during the first trimester of pregnancy (start after conception).

^c Early pregnancy: week 14 of pregnancy.

^d Late pregnancy: week 34 of pregnancy.

^e Low: primary school, preparatory vocational or lower general secondary school; middle: vocational, higher general secondary or pre-university education; and high: higher vocational or academic education.

**FIGURE 2**

Prevalence of wheeze (A) and eczema (B) at different ages.

and a power of 80%), our CI did not exclude the possibility of a true effect size of 1.06.

On the basis of a possible causal relationship between the methylenetetrahydrofolate reductase gene (*MTHFR*),

in particular the polymorphism *MTHFR* C677T, and allergic sensitization in Danish adults,⁹ impaired folate metabolism has been associated with the development of atopy. However, Granell et al¹⁶ and Thuesen et al¹⁷ found no evidence of an association between the *MTHFR* gene and the development of atopy or asthma in children.

To our knowledge, no previous study has investigated ICF levels in late pregnancy in relationship to the development of asthma and atopy in the offspring, but our results are in line with results from studies conducted in chil-

TABLE 2 Association Between Folic Acid Supplement Use During Pregnancy and Childhood Atopic Diseases

	Folic Acid Use		Folic Acid Use (Divided Into Categories)			
	No Use at All (Reference)	Yes	No Use at All (Reference)	During Early Pregnancy ^a	During Whole Pregnancy	During Another Period
Eczema until 6–7 y^b						
Crude OR (95% CI) ^c	1.0	1.20 (0.94 to 1.53)	1.0	1.14 (0.88 to 1.49)	1.34 (1.02 to 1.75)	1.12 (0.84 to 1.48)
aOR (95% CI)	1.0	1.16 (0.90 to 1.48)	1.0	1.12 (0.86 to 1.46)	1.27 (0.96 to 1.66)	1.08 (0.81 to 1.43)
AD at 2 y^d						
Prevalence, n/N (%)	16/136 (11.8)	97/706 (13.7)	16/136 (11.8)	40/305 (13.1)	35/234 (15.0)	22/167 (13.2)
Crude OR (95% CI) ^c	1.0	1.17 (0.66 to 2.06)	1.0	1.12 (0.60 to 2.08)	1.28 (0.68 to 2.43)	1.12 (0.56 to 2.23)
aOR (95% CI)	1.0	1.15 (0.63 to 2.10)	1.0	1.15 (0.60 to 2.20)	1.26 (0.60 to 2.63)	1.06 (0.50 to 2.21)
Increased total IgE at 2 y^{d,e}						
Prevalence, n/N (%)	54/131 (41.2)	215/676 (31.8)	54/131 (41.2)	99/295 (33.6)	70/222 (31.5)	46/159 (28.9)
Crude OR (95% CI) ^c	1.0	0.68 (0.46 to 0.99)	1.0	0.73 (0.48 to 1.11)	0.67 (0.43 to 1.06)	0.59 (0.36 to 0.96)
aOR (95% CI)	1.0	0.71 (0.47 to 1.07)	1.0	0.72 (0.46 to 1.12)	0.78 (0.46 to 1.32)	0.62 (0.37 to 1.05)
Increased specific IgE at 2 y^{d,f}						
Prevalence, n/N (%)	33/131 (25.2)	187/668 (28.0)	33/131 (25.2)	81/292 (27.7)	62/220 (28.2)	44/156 (28.2)
Crude OR (95% CI) ^c	1.0	1.12 (0.73 to 1.73)	1.0	1.12 (0.70 to 1.79)	1.11 (0.68 to 1.83)	1.14 (0.67 to 1.93)
aOR (95% CI)	1.0	1.06 (0.67 to 1.68)	1.0	1.02 (0.62 to 1.67)	1.11 (0.63 to 1.96)	1.13 (0.64 to 1.98)
Wheeze until 6–7 y^b						
Crude OR (95% CI) ^c	1.0	1.08 (0.87 to 1.34)	1.0	1.05 (0.83 to 1.33)	1.07 (0.84 to 1.35)	1.14 (0.89 to 1.45)
aOR (95% CI)	1.0	0.99 (0.80 to 1.23)	1.0	1.00 (0.79 to 1.26)	0.97 (0.76 to 1.23)	1.02 (0.80 to 1.30)
Asthma at 6–7 y^d						
Prevalence, n/N (%)	14/255 (5.5)	116/1647 (7.0)	14/255 (5.5)	44/624 (7.1)	41/607 (6.8)	31/416 (7.5)
Crude OR (95% CI) ^c	1.0	1.23 (0.69 to 2.18)	1.0	1.25 (0.67 to 2.33)	1.15 (0.61 to 2.16)	1.31 (0.68 to 2.51)
aOR (95% CI)	1.0	1.19 (0.65 to 2.20)	1.0	1.27 (0.67 to 2.41)	1.06 (0.51 to 2.17)	1.15 (0.57 to 2.32)
Lung function at 6–7 y^g						
%FEV ₁						
Crude β (95% CI) ^c	0.0	1.10 (−3.65 to 5.85)	0.0	2.21 (−2.84 to 7.26)	0.11 (−4.90 to 5.12)	0.27 (−5.28 to 5.82)
Adjusted β (95% CI)	0.0	0.55 (−4.50 to 5.61)	0.0	1.96 (−3.37 to 7.28)	−0.45 (−6.14 to 5.23)	−0.62 (−6.65 to 5.40)
%FVC						
Crude β (95% CI) ^c	0.0	1.26 (−3.03 to 5.55)	0.0	1.72 (−2.89 to 6.32)	1.82 (−2.74 to 6.39)	−0.81 (−5.87 to 4.25)
Adjusted β (95% CI)	0.0	0.34 (−4.21 to 4.90)	0.0	1.08 (−3.78 to 5.95)	1.02 (−4.17 to 6.21)	−1.95 (−7.45 to 3.56)

Models were adjusted for: maternal antibiotic use during pregnancy, maternal smoking during pregnancy, maternal alcohol consumption during pregnancy, mode and place of delivery, birth weight, gender of the child, treatment with antibiotics during the first 6 months of life, breastfeeding during the first 2 years of life, exposure to environmental tobacco smoke in the first 6 to 7 years of life, siblings, family history, recruitment group, maternal education level, day care, and other supplement use during pregnancy.

^a The use during the advice period (4 weeks before conception until 8 weeks after conception) and/or use solely during the first trimester of pregnancy (start after conception). The separate categories were not associated with any of the outcomes and were therefore combined in the analyses.

^b Using GEE analyses.

^c The crude OR and crude β were adjusted for recruitment group.

^d Using logistic regression analyses.

^e Increased total IgE refers to a total IgE >25.0 IU/mL (67th percentile of the distribution in the total cohort).

^f Increased specific IgE refers to at least 1 allergen-specific IgE >0.3 IU/mL.

^g Using linear regression analyses.

TABLE 3 Association Between IFC Levels During Pregnancy and Childhood Atopic Diseases

	IFC Levels (Divided Into Quintiles)					<i>P</i> for Trend ^a
	1st Quintile (≤480 nmol/L)	2nd Quintile (481–643 nmol/L)	3rd Quintile (644–862 nmol/L)	4th Quintile (863–1139 nmol/L)	5th Quintile (≥1140 nmol/L)	
Eczema until 6–7 y ^b						
crude OR (95% CI) ^c	1.0	1.35 (0.86 to 2.10)	1.08 (0.69 to 1.70)	1.24 (0.81 to 1.89)	1.48 (0.96 to 2.27)	.16
aOR (95% CI)	1.0	1.29 (0.83 to 2.01)	1.04 (0.65 to 1.67)	1.13 (0.74 to 1.74)	1.35 (0.87 to 2.09)	.36
AD at 2 y ^d						
Prevalence, <i>n/N</i> (%)	11/158 (7.0)	14/158 (8.9)	15/158 (9.5)	22/158 (13.9)	11/158 (7.0)	
Crude OR (95% CI) ^c	1.0	1.57 (0.67 to 3.66)	1.48 (0.64 to 3.40)	2.43 (1.11 to 5.33)	1.20 (0.49 to 2.93)	.31
aOR (95% CI)	1.0	1.32 (0.53 to 3.29)	1.44 (0.56 to 3.71)	2.25 (0.89 to 5.67)	0.94 (0.33 to 2.67)	.70
Increased total IgE at 2 y ^{d,e}						
Prevalence, <i>n/N</i> (%)	34/158 (21.5)	24/158 (15.2)	27/158 (17.1)	39/158 (24.7)	32/158 (20.3)	
Crude OR (95% CI) ^c	1.0	0.74 (0.39 to 1.38)	0.69 (0.38 to 1.27)	1.26 (0.71 to 2.24)	1.08 (0.59 to 1.97)	.33
aOR (95% CI)	1.0	0.75 (0.38 to 1.46)	0.87 (0.44 to 1.74)	1.79 (0.89 to 3.61)	1.33 (0.64 to 2.80)	.10
Allergic sensitization at 2 y ^{d,f}						
Prevalence, <i>n/N</i> (%)	33/158 (20.9)	17/158 (10.8)	29/158 (18.4)	28/158 (17.7)	24/158 (15.2)	
Crude OR (95% CI) ^c	1.0	0.52 (0.26 to 1.02)	0.86 (0.47 to 1.58)	0.84 (0.46 to 1.55)	0.81 (0.43 to 1.52)	.92
aOR (95% CI)	1.0	0.47 (0.23 to 0.99)	0.86 (0.43 to 1.71)	0.86 (0.42 to 1.78)	0.70 (0.32 to 1.51)	.81
Wheeze until 6–7 y ^b						
Crude OR (95% CI) ^c	1.0	0.89 (0.61 to 1.32)	0.69 (0.47 to 1.01)	0.78 (0.53 to 1.15)	0.98 (0.67 to 1.43)	.70
aOR (95% CI)	1.0	0.88 (0.60 to 1.29)	0.73 (0.51 to 1.06)	0.80 (0.54 to 1.19)	1.01 (0.69 to 1.49)	.90
Asthma at 6–7 y ^d						
Prevalence <i>n/N</i> (%)	13/158 (8.2)	10/158 (6.3)	7/158 (4.4)	7/158 (4.4)	6/158 (3.8)	
Crude OR (95% CI) ^c	1.0	0.84 (0.35 to 2.02)	0.52 (0.20 to 1.35)	0.51 (0.20 to 1.33)	0.44 (0.16 to 1.21)	.05
aOR (95% CI)	1.0	0.73 (0.27 to 1.94)	0.46 (0.15 to 1.41)	0.41 (0.12 to 1.34)	0.31 (0.09 to 1.10)	.05
Lung function at 6–7 y ^g						
%FEV ₁						
Crude β (95% CI) ^c	0.0	1.63 (−4.64 to 7.89)	−1.99 (−7.69 to 3.71)	−1.55 (−7.23 to 4.12)	1.23 (−4.58 to 7.31)	.90
Adjusted β (95% CI)	0.0	0.25 (−6.55 to 7.06)	−2.58 (−8.74 to 3.58)	−2.97 (−9.27 to 3.33)	−0.92 (−6.82 to 6.64)	.61
%FVC						
Crude β (95% CI) ^c	0.0	1.78 (−3.93 to 7.48)	−0.56 (−5.75 to 4.63)	−1.40 (−5.30 to 5.02)	1.08 (−4.45 to 6.62)	.94
Adjusted β (95% CI)	0.0	1.13 (−4.97 to 7.24)	−1.36 (−6.88 to 4.17)	−1.19 (−6.84 to 4.46)	−0.65 (−6.68 to 5.39)	.61

Models were adjusted for: maternal antibiotic use during pregnancy, maternal smoking during pregnancy, maternal alcohol consumption during pregnancy, mode and place of delivery, birth weight, gender of the child, treatment with antibiotics during the first 6 months of life, breastfeeding duration, exposure to domestic animals during pregnancy and the first 2 years of life, exposure to environmental tobacco smoke in the first 6 to 7 years of life, siblings, family history of atopy, recruitment group, maternal education level, day care, and multivitamin or other supplement use during pregnancy. Due to too-small numbers in the categories siblings unknown, low birth weight, and unknown mode and place of delivery, these categories were recoded into the reference group.

^a Test for trend over quintiles (coded 1–5).

^b Using GEE analyses.

^c The crude OR and crude β were adjusted for recruitment group.

^d Using logistic regression analyses.

^e Increased total IgE refers to a total IgE >25.0 IU/mL (67th percentile of the distribution in the total cohort).

^f Allergic sensitization refers to at least 1 allergen-specific IgE >0.3 IU/mL.

^g Using linear regression analyses.

dren and adults aged 2 to 60 years.^{10,15} However, different mechanisms may play a role during pregnancy than after birth, making it difficult to compare the results of these studies with those of our study. A lower folic acid level has been associated with physician-diagnosed asthma but not with sensitization or lung function in cross-sectional analyses.¹⁵ Matsui and Matsui¹⁰ found that serum folic acid levels were inversely associated with sensitization, wheeze, and physician-diagnosed asthma, although the latter

failed to reach statistical significance. Recently, Håberg et al¹⁸ analyzed maternal plasma folate levels in week 18 of pregnancy and found that there was a trend of increasing risk for developing asthma in the offspring at age 3 years across quintiles of plasma folate (*P* trend = .006).

We did not consider dietary folic acid intake. Most green leafy vegetables and fruit contain folic acid but in very small amounts.³⁰ Moreover, 54% of the Dutch women do not meet the current

Dutch folic acid recommendations of 200 μ g per day,³⁰ and the bioavailability of folic acid from food (not fortified) is 50% less than from supplements.^{30–32} Folic acid content of supplements used by women in the studies of Håberg et al¹ and Whitrow et al¹⁴ (400 and 300 μ g, respectively) was comparable to that of our study (400 μ g). Similar to the Netherlands,³³ countries in which the previous studies^{1,14} were conducted do not fortify food with folic acid. However, there seems to be some controversy about this.³⁴

The main strength of our study lies in the long prospective follow-up (6–7 years) with relatively short intervals between follow-up questionnaires, which should decrease recall bias.³⁵ Follow-up rates were high, and study populations' characteristics at 6 to 7 years of age were similar to those at birth, making bias as result of differential follow-up unlikely. Bias due to different recruitment channels is also unlikely, given that the interaction between exposure and recruitment group was not statistically significant. Furthermore, we were able to adjust for a large number of potential confounders. Because we adjusted for multivitamin supplement use and were able to investigate folic acid use as a stand-alone supplement, it is highly unlikely that our results were confounded by other vitamins used.

A potential limitation is that missing data due to nonresponse could have had some influence on our results, but we expect this to be nondifferential, at most biasing our results toward null. Often, missingness is associated with socioeconomic status. Because such data were not collected, educational

level of the mother was used as proxy for socioeconomic information. Supplemental Table 4 shows that the educational level of the mother was not associated with missingness. The ISAAC questions, widely used in birth cohort studies for assessing wheeze and eczema, have been validated for children aged 6 to 7 years but not in younger children. This factor could have led to misclassification. Different definitions are used for wheeze and asthma, but they will inherently overlap because wheeze is the main symptom of asthma. The same holds true for eczema and AD. Furthermore, considering the quantity of analyses performed in the present study and an α level of 5%, 6 or 7 statistically significant associations could be based on chance alone.

Folic acid concentration measured in red blood cells provides an indication of the folic acid status in the preceding 3 months.³² Because blood samples were taken in week 35 of pregnancy, no conclusions can be drawn about the relationship between ICF levels during early pregnancy and the development of childhood atopic manifestations.

CONCLUSIONS

Our results do not confirm any meaningful association between folic acid supplement use during pregnancy and atopic diseases in the offspring. Higher ICF levels in late pregnancy tended, at most, toward a small decreased risk for developing asthma. However, more research on folic acid use and ICF levels during pregnancy is warranted.

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Folic Acid Use in Pregnancy and the Development of Atopy, Asthma, and Lung Function in Childhood

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