

Relationship Between Prenatal and Postnatal Exposures to Folate and Risks of Allergic and Respiratory Diseases in Early Childhood

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Summary. Background: It is uncertain whether folate is risk or preventive factor for allergic and respiratory diseases. Objective: To determine the relationship between maternal or offspring folate status and subsequent development of allergic and respiratory diseases in early childhood. Methods: In total, 917 mother-child pairs from a prospective birth cohort in South Korea were studied. Data regarding the children's allergic and respiratory outcomes were obtained from standardized questionnaires completed by the mothers at postnatal months 6, 12, and 24. Serum folate levels were measured in the mothers at mid- and late-pregnancy, and in their children at 24 months of age. Atopic biomarkers were measured in the cord blood (CB) and at 24 months after birth. Biomarkers and clinical outcomes were analyzed and compared between the mother-child pairs divided into two groups according to median serum folate status at mid- and late-pregnancy. Results: Serum folate levels during mid-pregnancy were inversely associated with CB eosinophil count (adjusted odds ratio [OR] 0.72, 95% confidence interval [CI], 0.54–0.96) and positively associated with CB interleukin-10 levels (1.47, 1.11–1.94). Maternal folate level above the median value (≥ 9.5 ng/ml) during mid-pregnancy was associated with a decreased risk for the child of lower respiratory tract infections (LRTIs) at 6 months of age (adjusted OR 0.50, 95% CI 0.28–0.91) and atopic dermatitis (AD) at 24 months (adjusted OR 0.52, 95% CI 0.31–0.88), but not with LRTIs and AD at other ages. Conclusions: A relatively high maternal serum folate level in mid-pregnancy was associated with a decreased risk of LRTIs and AD in early childhood. **Pediatr Pulmonol.**

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INTRODUCTION

There is evidence from recent studies that exposure during the intrauterine period to certain factors such as the maternal diet, pollutants, microbes, and smoking influences the programming of the fetal immune system in favor of the development of allergic diseases.^{1–3} Epigenetic changes in gene expression could explain how prenatal exposure to environmental factors affects disease risk later in life.^{1–3}

Dietary folate is the most extensively studied micronutrient, particularly in the field of epidemiologic research. Although there is considerable evidence that maternal folate supplementation prevents neural-tube defects and other congenital anomalies,^{4,5} there are conflicting data regarding whether dietary folate intake or folic acid supplementation induces the development of allergic or respiratory diseases.

The folate hypothesis for allergic disease is based on epigenetic mechanisms that may influence gene expression, thus regulating allergic inflammation.^{1–3} The results of some epidemiologic studies involving humans^{6–8} and one animal study⁹ suggest that taking folic acid supplements during pregnancy is associated with an increased risk of asthma or asthma-like symptoms^{6–8} and allergic airway inflammation in the offspring.⁹ Two studies also found an increased risk of eczema in the offspring of mothers using folic acid supplements during pregnancy.^{10,11} However, some other studies involving adults have found that impaired folate metabolism or folate deficiency is a risk factor of the development of atopy¹² or asthma.^{13–15} On the other hand, the small number of studies investigating the association between folic acid supplements during pregnancy and lower respiratory tract infections (LRTIs) and risk of allergic disease have yielded inconsistent results.^{7,16,17} Therefore, clarification of the benefits and risks of folate supplements to allergic and respiratory diseases is imperative to guide public health policies.

To the best of our knowledge, no previous studies have measured maternal folate during pregnancy followed by the folate status of the offspring in order to evaluate the effects of maternal folate levels on allergic or respiratory outcomes in offspring. Therefore, the aim of this study was to determine the association between serum folate levels during pregnancy and in the resulting offspring, and atopic biomarkers and the risk of developing allergic disease and respiratory infection in the offspring up to 24 months after birth.

MATERIALS AND METHODS

Study Design and Population

The study population comprised pregnant women and their children from the Mothers and Children's Environmental Health (MOCEH) study in South Korea. MOCEH is a multicenter birth cohort study that was designed to investigate the effects of pre- and post-environmental exposures on pregnant women, early fetal life, and early childhood health outcomes. A detailed description of the study has been published previously.¹⁸

Of the 1,824 pregnant women who were recruited between August 2006 and October 2011, those with the following characteristics were excluded from the analysis: delivery before 37 weeks of gestational age (80 cases), and pregnancy complications including hypertension, gestational diabetes, or intrauterine growth retardation, twins, and congenital anomaly (28 cases). Of the remaining 1,716 subjects, 799 were also excluded because their data on serum folate and on atopic biomarkers were incomplete. Therefore, the data available for analysis were based on 917 pregnant women-child pairs (53.4% of the initial cohort).

All mothers and their offspring were monitored prospectively; 666 (72.6%), 595 (64.9%), and 462 (50.4%) of them left in the study when the child reached the age of 6, 12, and 24 months, respectively (Fig. 1). The study protocol was approved by the institutional review boards of Ewha Womans University School of Medicine (approval no. 12–07B-15), Dankook University Hospital (approval no. 2011–09–0340), and Ulsan University Hospital (approval no. 06–29), and written informed consent to participate was obtained from all of the mothers.

Dietary Assessment

The mothers' dietary intake data were assessed using an extensive food frequency questionnaire; details of the

ABBREVIATIONS:

AD	atopic dermatitis
CB	cord blood
IL-10	interleukin 10
LOD	limit of detection
LRTIs	lower respiratory tract infections
MOCEH	Mothers and Children's Environmental Health

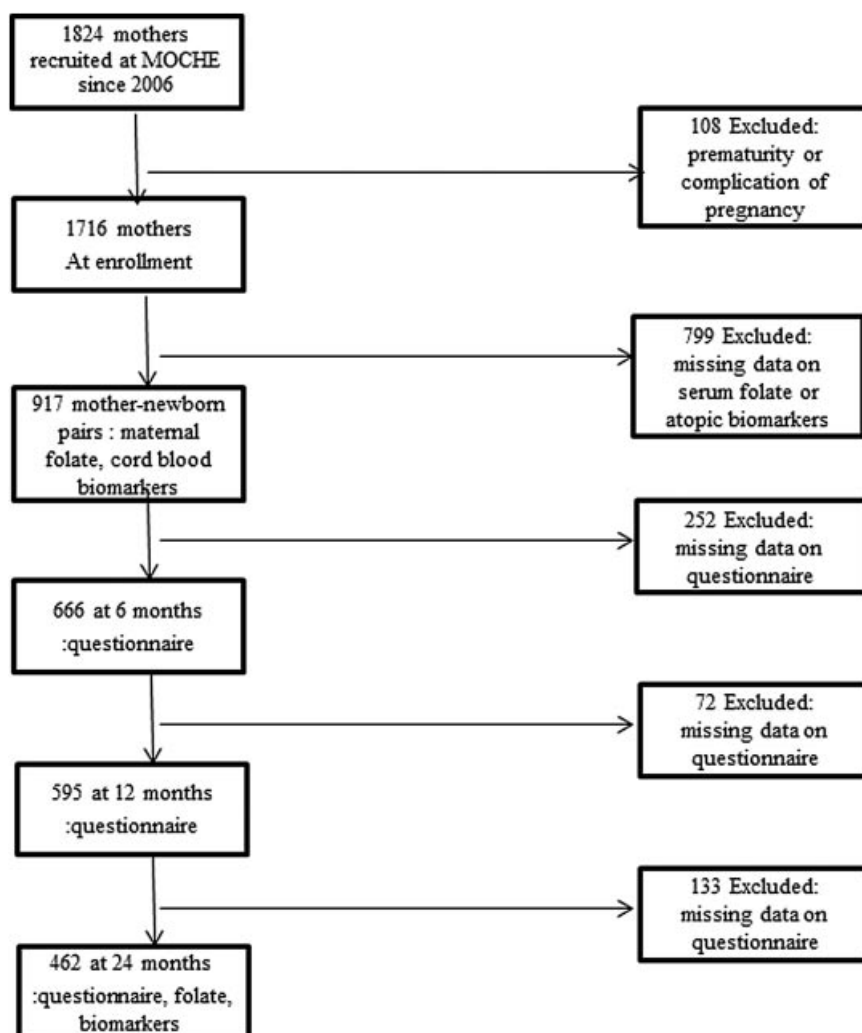


Fig. 1. Flow chart illustrating participants.

dietary assessment procedures are available elsewhere.¹⁹ Data regarding dietary folate intake, including the use of supplements containing folic acid, were obtained by well-trained dietary interviewers using a 24-hr recall protocol, and estimated using a nutrient-intake assessment program (CAN-Pro3.0, Korean Nutrition Society, Seoul, Korea).

Determination of Serum Folate Levels and Atopic Biomarkers

Venous blood samples were drawn from the mothers after a 12-hr overnight fast during mid-pregnancy (12–28 gestational weeks) and late-pregnancy (29–42 gestational weeks), and stored at -70°C until analysis. Serum folate concentration in these samples was determined using the SimulTRAC-SNB radioassay kit (MP Biomedicals, Orangeburg, NY), following the supplier's instructions.

Serum total IgE, eosinophil count, and interleukin (IL)-10 (as an atopic biomarker) level were measured in cord blood (CB) samples and in the samples taken from the children at age 24 months, as was serum folate in venous samples. CB total IgE levels were measured using commercially available paper radioimmunosorbent test kits (Pharmacia Diagnostics, Uppsala, Sweden); the limit of detection (LOD) for CB total IgE was 0.5 pg/ml. Blood eosinophils were counted using an automatic blood cell counter (XE-2100, Sysmex, Tokyo, Japan). IL-10 was measured using a quantitative sandwich enzyme immunoassay technique (Quantikine, Human IL-10 Immunoassay, R&D System, Minneapolis, MN); the LOD for IL-10 was 1 pg/ml. The level of cotinine in maternal urine was used as a marker for exposure to nicotine. Urine (100 μl) was collected in mid- and late-pregnancy and stored at -20°C for later analysis. Quantitative urinary cotinine was analyzed with the Calbiotech Cotinine

enzyme-linked immunosorbent assay (Calbiotech, Spring Valley, CA) following the manufacturer's protocols. The LOD of the cotinine assay was 1 ng/ml.

Outcome Assessment

All participants were asked to complete a structured questionnaire to obtain demographic and socioeconomic information at enrollment. Postnatal data on allergic disease and respiratory infections were collected by questionnaire when the child reached 6, 12, and 24 months of age. A modified version of the International Study of Asthma and Allergies in Childhood questionnaire²⁰ was used to obtain information on asthma and atopic dermatitis (AD).

The following three outcomes were defined, and assessed at three time points: when the child reached 6, 12, and 24 months. AD was considered to be present when there was a positive answer to the question "Did a doctor ever diagnose AD in your child?" or "Has your child ever visited hospital for treatment of AD in the last 12 months?" Asthma was considered to be present when there was a positive answer to the question "Did a doctor ever diagnose asthma in your child?" or "Has your child ever visited hospital for treatment of asthma in the last 12 months?" An LRTI was considered to be present when there was a positive answer to the question "Has a doctor ever said that your child has bronchitis, bronchiolitis, or pneumonia?" or "Has your child ever visited hospital for treatment of bronchitis, bronchiolitis, or pneumonia in the last 12 months?"

Statistical Analyses

All data are expressed as mean $\pm$ standard deviation (for continuous variables) or number and percentage (for categorical variables) values. Folate variables were not normally distributed, and so the difference in atopic biomarkers and clinical outcomes based on their folate level was examined by dividing the mothers into two groups according to the cohort's median folate levels at mid- and late-pregnancy. Independent Student's *t*-test and chi-square test were used to compare demographic differences between these two groups.

Before we determine the association between the serum folate levels and atopic biomarkers, maternal folate levels during pregnancy and atopic biomarkers (including Total IgE, eosinophil count, and IL-10) were dichotomized into low and high as independent and dependent variables each according to median value. We performed logistic regression to estimate odds ratios (ORs) and their 95% confidence intervals (CIs) for total IgE, eosinophil count, and IL-10 according to folate group after adjustment for covariates.

Logistic regression analysis was also performed to estimate ORs and their 95% CIs for AD, asthma, and

LRTIs in relation to folate levels during pregnancy and in the children. Multiple logistic regression analysis was employed to adjust for potential confounding factors. All models were adjusted for the baby's sex, birth weight, gestational age, and duration of breastfeeding, and maternal age, the history of allergic diseases such as AD, asthma, allergic rhinitis, and allergic conjunctivitis, the urinary cotinine levels in mid- and late-pregnancy, and the prepregnancy body mass index (BMI). Two-sided *P*-values less than 0.05 were considered statistically significant. All statistical analyses were performed using SPSS 19.0 (SPSS, Chicago, IL).

RESULTS

Baseline Characteristics of the Cohort

The baseline characteristics of the mothers and children included in this study are given in Table 1. The dietary folate uptake during pregnancy was $354.9 \pm 140.0 \mu\text{g}$, and more than half (62.3%) of the mothers took folic acid supplements. The folates levels during mid- and late-pregnancy were 11.5 ± 8.6 and $13.3 \pm 9.5 \text{ ng/ml}$, respectively. A folate level of $\geq 6 \text{ ng/ml}$ was found at mid- and late-pregnancy in 75.0% and 78.6% of the pregnant women, respectively.

Prevalence of Allergic and Respiratory Outcomes

The prevalence of allergic and respiratory outcomes among the children at 6–24 months of age is shown in Table 2. The prevalence of AD was relatively high, ranging from 25.5% to 28.7% up to 24 months of age. However, the asthma prevalence was relatively low, ranging from 2.7% to 4.3%. The prevalence rates of LRTIs among the children were 13.2%, 20.1%, and 26.6% at 6, 12, and 24 months of age, respectively.

Association Between Folate Levels and Atopic Biomarkers

The mothers were divided into low- and high-folate groups according to the median folate value during mid-pregnancy (i.e., <9.5 and $\geq 9.5 \text{ ng/ml}$), and atopic biomarkers were also categorized as low and high according to median value. The relationship between folate level and atopic biomarkers was compared between the two groups (Table 3). The CB IL-10 level was significantly higher in the high-folate group than in the low-folate group, whereas there were no group differences in total IgE or eosinophil count. The mothers were also divided into low- and high-folate groups according to their serum folate level during late-pregnancy using the median value (i.e., <11.2 and $\geq 11.2 \text{ ng/ml}$) and their data analyzed and compared. However, none of the biomarkers differed significantly between the two groups (data not shown) and there were also no significant group

TABLE 1—Baseline Demographics of the MOCHE Birth Cohort

Characteristics	Total
	N (%) or mean SD
Mothers characteristics	
Age (year)	30.2 (3.7)
Prepregnancy BMI (kg/m ²)	21.7 (3.2)
Folate intake (ug/day)	354.85 (140.06)
Maternal educational levels	
≤High school	220 (24)
College or university	584 (63.7)
>University	57 (6.2)
Unknown	56 (6.1)
Maternal history of allergic disease	
Yes	665 (76)
No	210 (24)
Family smoking	
Yes	272 (42.8)
No	363 (57.2)
Serum folate (ng/ml)	
At mid-pregnancy	11.5 (8.6)
At late-pregnancy	13.3 (9.5)
Urine cotinine (ng/ml)	
At mid-pregnancy	52.1 (501.2)
At late-pregnancy	43.2 (440.3)
Children characteristics	
Male sex	477 (52)
Birth weight (g)	3185.2 (730.1)
Gestational age (weeks)	39.1 (1.1)
Breastfeeding (months)	8.3 (4.6)
Folate (ng/ml) at 24 months	20.9 (12.5)
Atopic biomarker	
Cord blood	
Total IgE (IU/ml)	1.7 (5.7)
Eosinophil count (/mm ³)	387.9 (497)
IL-10 (pg/ml)	1.3 (3.2)
24 months	
Total IgE (IU/ml)	90.1 (281.5)
Eosinophil count (/mm ³)	231.5 (217.1)
IL-10 (pg/ml)	2.2 (5.3)

differences in the children's biomarkers (Table 3). These findings prompted the focus of the subsequent analysis to shift to serum folate levels during mid-pregnancy.

The results of ORs and 95% CI for total IgE, eosinophil count, and IL-10 according to folate group are reported in Table 4. In logistic regression analysis using folate group as an independent variable after covariate adjustment, high-folate group (≥ 9.4 ng/ml), compared to low-folate

group (< 9.4 ng/ml) at mid-pregnancy, had a lower risk (OR 0.72, 95% CI 0.54–0.96) of having high eosinophil count in CB. Furthermore high-folate group at mid and late-pregnancy was significantly associated with increased IL-10 levels in CB (OR 1.47, 95% CI 1.11–1.94; OR 2.01, 95% CI 1.52–2.66), even after adjustment of covariates. However, there was no association between folate levels during mid- and late-pregnancy and the child's atopic biomarkers at 24 months.

Relationship Between Folate Group and Allergic or Respiratory Outcomes

The crude and adjusted ORs from multiple logistic regression analyses for the respiratory or allergic outcomes in the children up to the age of 24 months according to folate group are given in Table 5. The association between folate levels and allergic outcomes varied with the pregnancy stage. There was no association between the folate group and risk of AD in the children at 6 and 12 months of age. However, at mid-pregnancy there was an inverse relationship between the risk of AD in the children at 24 months and high-folate levels (≥ 9.5 ng/ml) compared to low-folate levels (< 9.5 ng/ml); this association persisted after adjusting for covariates (adjusted OR 0.52, 95% CI 0.31–0.88, $P = 0.014$). In late-pregnancy, there was no association between folate level and risk of AD or LRTIs in the children at 6, 12, or 24 months of age. We found that the folate level with AD was lower than that without AD in the children at 24 months, but no associations were observed between child's folate level and AD at 24 months (data not shown).

A decreased risk of LRTIs in the children at 6 months of age was associated with high-folate at mid-pregnancy (adjusted OR 0.50, 95% CI 0.28–0.91, $P = 0.022$; Table 5) after adjusting for the maternal age, BMI, history of allergic disease, and urinary cotinine level at mid-pregnancy, and the baby's sex, gestational age, and duration of breastfeeding. However, there was no significant association between folate levels and LRTIs at older ages (Table 5).

DISCUSSION

In this Korean population, the prevalence of AD at 24 months and LRTIs at 6 months were significantly lower in the children of mothers with serum folate levels above the median (9.5 ng/ml) in mid-pregnancy. However, no association between folate levels and allergic or respiratory outcomes was seen in late-pregnancy or in the children. These findings suggest that folate levels are more important during mid-pregnancy rather during late-pregnancy, and higher levels of maternal serum folate are needed to decrease the risk of allergic or respiratory outcomes.

TABLE 2—The Prevalence of Allergic and Respiratory Outcomes From 6 to 24 Months of Age

Outcome	6 months	12 months	24 months
AD	191/666 (28.7%)	146/595 (24.5%)	123/462 (25.5%)
Asthma		16/595 (2.7%)	20/462 (4.3%)
LRTIs	88/666 (13.2%)	120/595 (20.1%)	123/462 (26.6%)

TABLE 3— Association of Maternal Mid-Pregnancy Folate Group and Atopic Biomarkers

		Total	Low-folate	High-folate	
Atopic biomarker	Level	(n = 917)	(n = 451) (n, %)	(n = 466) (n, %)	<i>P</i> -value
Cord blood					
Total IgE	Low	463 (50.5)	233 (51.7)	230 (49.4)	0.485
	High	454 (49.5)	218 (48.3)	236 (50.6)	
Eosinophil count	Low	389 (52.6)	186 (53.1)	203 (52.1)	0.767
	High	351 (47.4)	164 (46.9)	187 (47.9)	
IL-10	Low	586 (63.9)	314 (69.6)	272 (58.4)	0.000
	High	331 (36.1)	137 (30.4)	194 (41.6)	
24 months					
Total IgE	Low	168 (49.7)	82 (48.2)	86 (51.2)	0.587
	High	170 (50.3)	88 (51.8)	82 (48.8)	
Eosinophil count	Low	170 (50.4)	90 (52.3)	80 (48.5)	0.481
	High	167 (49.6)	82 (47.7)	85 (51.5)	
IL-10	Low	162 (53.8)	82 (49.1)	80 (59.7)	0.067
	High	139 (46.2)	85 (50.9)	54 (40.3)	

The endogenous production of IgE and a dramatic rise in IgE receptor activity in the fetus were found to occur from the second trimester.²¹ Although inconsistent with our findings, Haberg et al.⁸ reported that folate levels in the second trimester of pregnancy were positively associated with the risk of developing asthma in children at 3 years. With regard to exposure time, it can thus be speculated that mid-pregnancy is the most critical time for the effect of folate on the development of allergic diseases.

In addition to the timing of exposure to environmental factors, it is important to determine the optimal value of maternal serum folate levels during pregnancy for maximizing outcomes in the offspring. Normal serum

folate levels were varied depending on trimester and were considered as follows; first trimester (2.6–15 ng/ml), second trimester (0.8–24 ng/ml), and third trimester (1.4–20.7 ng/ml).²² In this study, 75.0% and 78.6% of pregnant women had folate levels of ≥ 6 ng/ml at mid- and late-pregnancy, respectively. The risk of AD was decreased for folate levels above the median value (9.5 ng/ml) during mid-pregnancy in children aged 24 months, after adjusting for potential confounders. This finding suggests that the prevalence of serum folate deficiency is relatively low in South Korea. A recent study found that about 63% of the pregnant women living in South Korea took supplements containing both iron and folic acid.²³ Similarly, in

TABLE 4— ORs and 95% CIs for Total Ig E, Eosinophil Count, and IL-10 According to Folate Group at Mid- and Late-Pregnancy

Pregnancy	Folate group	Total IgE (CB)	Eosinophil count (CB)	IL-10 (CB)
		OR (95% CI)	OR (95% CI)	OR (95% CI)
Middle	Low	1	1	1
	High	1.11 (0.85–1.44)	0.72 (0.54–0.96)	1.47 (1.11–1.94)
Late	Low	1	1	1
	High	0.95 (0.73–1.23)	1.13 (0.85–1.52)	2.01 (1.52–2.66)
		Total IgE (24 months)	Eosinophil count (24 months)	IL-10 (24 months)
		OR (95% CI)	OR (95% CI)	OR (95% CI)
Middle	Low	1	1	1
	High	0.85 (0.55–1.30)	0.97 (0.63–1.50)	0.69 (0.44–1.09)
Late	Low	1	1	1
	High	1.29 (0.84–1.98)	1.17 (0.76–1.80)	1.05 (0.66–1.65)

All atopic biomarker were categorized according to median value (high vs. low).

The bold text indicates statistically significant results.

Covariates adjusted in the model: birth weight, gestational age, and duration of breastfeeding, and maternal age, maternal history of allergic disease, urinary cotinine levels in mid- and late-pregnancy, and prepregnancy BMI.

TABLE 5—Association of Maternal Mid- and Late-Pregnancy Folate and Allergic or Respiratory Outcomes

Outcome		Mid-pregnancy folate			Late-pregnancy folate		
		OR	95% CI	P-value	OR	95% CI	P-value
6 months							
AD	Crude	0.92	0.65–1.29	0.639	1.28	0.91–1.80	0.153
	Adjusted	0.96	0.65–1.43	0.835	1.12	0.75–1.68	0.569
LRTIs	Crude	0.73	0.46–1.16	0.183	0.75	0.47–1.19	0.218
	Adjusted	0.50	0.28–0.91	0.022	0.64	0.36–1.16	0.143
12 months							
AD	Crude	0.87	0.60–1.27	0.48	1.32	0.91–1.93	0.148
	Adjusted	0.96	0.63–1.48	0.862	1.24	0.80–1.92	0.336
LRTIs	Crude	0.85	0.56–1.29	0.449	0.86	0.57–1.31	0.488
	Adjusted	0.81	0.50–1.30	0.379	0.75	0.47–1.21	0.243
24 months							
AD	Crude	0.62	0.41–0.96	0.030	1.26	0.82–1.92	0.289
	Adjusted	0.52	0.31–0.88	0.014	1.4	0.85–2.32	0.190
LRTIs	Crude	0.80	0.52–1.22	0.297	0.96	0.63–1.47	0.857
	Adjusted	0.72	0.43–1.21	0.219	1.25	0.75–2.07	0.389

The bold text indicates statistically significant results.

Maternal folate model was adjusted: birth weight, gestational age, and duration of breastfeeding, and maternal age, maternal history of allergic disease, urinary cotinine levels in mid- and late-pregnancy, and prepregnancy BMI.

Low-folate group was used as the reference group for the regression.

the USA approximately 77% of pregnant women reportedly take supplements containing folic acid.²⁴ However, in other studies the risk of allergic disease was associated with relatively high folate levels, such as >17.8 nmol/l⁸ or >16.2 nmol/l,¹⁰ and a methyl-rich diet¹³ during pregnancy. Dunstan et al. recently suggested that the optimal range of CB folate levels for minimizing allergic sensitization was 50–75 mmol/l.¹¹ These findings indicate that it is necessary to determine the appropriate folate level during pregnancy that minimizes the potential risk of the child developing allergic diseases and to maintain the beneficial effects of preventing neural-tube defects.

The association between the child's serum folate level and allergic and respiratory outcomes was also evaluated in this study in order to determine the most important period (i.e., prenatal or postnatal) for exposure to folate with respect to avoidance of allergic and respiratory diseases. However, in the current study there were no associations between the folate level in the children and AD or LRTIs. In contrast to the our results, two studies involving adults have shown that higher serum folate levels are associated with a lower risk of atopy and wheeze,¹³ and that lower serum folate levels are associated with the presence of atopic asthma.¹⁵ A study involving Indian children found that folate deficiency was an independent risk factor for LRTIs.¹⁷ So the relationship between the folate status of children and allergic and respiratory outcomes remains to be established.

The association between serum folate levels during pregnancy or in children and atopic biomarkers in children was evaluated in this study with the aim of

elucidating the underlying mechanism leading to the allergic and respiratory diseases. Although it has been proposed that methyl donors (including folate) during pregnancy lead to alterations in gene methylation and allergic disease in the offspring via epigenetic mechanisms, further investigations are required to clarify the potential mechanism.^{1–3} Based on ORs in the present study, folate levels in mid-pregnancy were negatively associated with the eosinophil count and were positively associated with IL-10 level in CB. Folate levels may therefore be related to the development of allergic disease via IL-10, which is a widely accepted to act as a immunoregulator during infection or T helper (Th)1 Th2 cell responses.^{25–27} Previous studies have found that the decreased allergen-induced IL-10 production in atopic children may facilitate the development of atopic disease by a lack of down-regulation of the inflammatory process during the first 6 months of life,²⁵ and that IL-10 levels in the nasal secretions are significantly lower in infants with atopy and respiratory syncytial virus bronchiolitis than in those without atopy.⁷ In addition, there is some evidence that an inadequate folate status influences the humoral and cell-mediated immune system. Folate deficiency has been shown to reduce the proportion of circulating T lymphocytes and the response to mitogens, and to decrease the antibody responses to several antigens, leading to infection.^{28,29} IL-10 may therefore play an important role in the development of allergic or respiratory disease via both epigenetic and nonepigenetic mechanisms. However, further study is required to ascertain the roles of both IL-10 and folate on the development of allergic or respiratory diseases.

Some limitations should be considered when interpreting the findings of this study. First, CB folate levels were not measured, and other vitamins, as cofactors were not considered. Second, asthma data were excluded from the analysis because of a lack of statistical power resulting from the low asthma prevalence. This low prevalence may have been due to the difficulty in defining asthma in infants or the misclassification of LRTIs due to a reliance on maternal reports. Third, 462 out of the enrolled 1,716 were eligible. We considered that there would be possibly some substantial biases in our analyses due to the missing data from nonresponses. However, there was a minimal statistical difference in only educational level between the respondents and the nonrespondents. Finally, all outcomes were assessed by only maternal reports, which might lead to under- or over estimation of diagnosis of the outcomes.

So future studies should attempt to objectively measure allergic disease.

The strengths of our study include its prospective design and the previous studies providing little information on the folate status in infants or early childhood. Given that a significant mother-to-child correlation in cytokine-producing capacity has been detected in infants,³⁰ the relationships between folate levels in both the mother and child, and biological markers and clinical outcomes were investigated. Moreover, folate levels were measured at three times—mid- and late-pregnancy, and in the children—to identify at which point the exposure to folate is most likely to be protective for AD and LRTIs. Finally, many potential confounders were considered when assessing the independent effect of folate on clinical outcomes, including the urinary cotinine level.^{31,32}

In conclusion, the findings of this study show that it is more important to have relatively higher maternal serum folate levels in mid-pregnancy than at any other period in order to reduce the risk of AD and LRTIs in early childhood. However, further prospective studies should attempt to define the role of folate in pediatric allergic and respiratory health outcomes.

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