

The relationship between maternal folate status in pregnancy, cord blood folate levels, and allergic outcomes in early childhood

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Abstract

Background: Dietary changes may epigenetically modify fetal gene expression during critical periods of development to potentially influence disease susceptibility. This study examined whether maternal and/or fetal folate status in pregnancy is associated with infant allergic outcomes.

Methods: Pregnant women ($n = 628$) were recruited in the last trimester of pregnancy. Folate status determined by both food frequency questionnaires and folate levels in maternal and cord blood serum was examined in relation to infant allergic outcomes at 1 year of age ($n = 484$).

Results: Infants who developed allergic disease (namely eczema) did not show any differences in cord blood or maternal folate levels compared with children without disease. Although maternal folate intake from foods was also not different, folate derived from supplements was higher ($P = 0.017$) in children with subsequent eczema. Furthermore, infants exposed to $> 500 \mu\text{g}$ folic acid/day as a supplement *in utero* were more likely to develop eczema than those taking $< 200 \mu\text{g/day}$ (OR [odds ratio] = 1.85; 95% CI 1.14–3.02; $P = 0.013$), remaining significant after adjustment for maternal allergy and other confounders. There was a nonlinear relationship between cord blood folate and sensitization, with folate levels $< 50 \text{ nmol/l}$ (OR = 3.02; 95% CI 1.16–7.87; $P = 0.024$) and $> 75 \text{ nmol/l}$ (OR = 3.59; 95% CI 1.40–9.20; $P = 0.008$) associated with greater sensitization risk than levels between 50 and 75 nmol/l.

Conclusion: Fetal levels between 50 and 75 nmol/l appeared optimal for minimizing sensitization. While folate taken as a supplement in higher doses during the third trimester was associated with eczema, there was no effect on other allergic outcomes including sensitization. Further studies are needed to determine the significance of this.

Background

Changing maternal nutrition in pregnancy is among the leading candidates in the rise in a range of inflammatory diseases with Western environmental change (1). While this has impli-

Abbreviations

DfE, dietary folate equivalent units; DOHaD, developmental origins of health and disease; RDI, recommended daily intakes; SQ FFQ, semi-quantitative food frequency questionnaire.

cations for the 'developmental origins' of many diseases, the progressive rise in immune diseases, most notably allergic disease, has generated intense interest in the effects of modern dietary patterns on the developing immune system (2). In response to a declining intake of a range of nutrients in modern diets, it has become a routine to recommend vitamin supplements such as folic acid in pregnancy to improve pregnancy outcomes (3). However, while this initiative was directed at reducing the risk of neural tube defects, experimental data in mice (4) and observational data in humans have

suggested possible adverse effects of folic acid supplements in pregnancy on respiratory and allergic outcomes in offspring (5, 6).

A strong basis for this speculation comes from an animal model that demonstrated the potential epigenetic effects of folate as a dietary methyl donor, altering heritable gene expression through DNA methylation to induce experimental asthma and associated immune effects on the offspring (4). However, while this has raised question over public health policy (7), as yet, these findings are far from definitive. So far, human studies have been based on infant outcomes from questionnaire data and hospitalizations without allergy testing (5, 6, 8). The initial reports were also based only on questionnaire data collected on maternal folate intake from supplements (5, 6) and from background diet (6), also with recognized limitations. While a more recent follow-up report on one of these cohorts (8) showed consistent findings based on maternal serum folate at 18 weeks gestation, there was no biological measure of folate status in the infants.

Here, we examine the relationship between folate status in pregnant women and their neonates, and infants' allergic outcomes using more accurate measures of infant allergic outcomes based on detailed clinical phenotyping and skin prick testing (SPT). We have also performed a more detailed biological assessment of not only maternal levels but also neonatal folate status.

Methods

Recruitment of study population

The study population comprised 647 pregnant women in Western Australia recruited to investigate the effects of early dietary exposures on allergic outcomes, as approved by the Princess Margaret Hospital Ethics Committee. All participants (mothers) gave written informed consent. Only healthy nonsmokers with uncomplicated term pregnancies were included ($n = 628$). Blood samples and semi-quantitative food frequency questionnaires (SQFFQ) were collected in the third trimester (after 28 weeks gestation). The follow-up assessments for allergic outcomes occurred at 12 months of age. Some of the infants included in this analysis were included in a postnatal intervention with fish oil (9). However, this intervention had no clinical effects on allergic outcomes, and there is also no evidence to suggest that this would have had any effect on folate status (10, 11). Nonetheless, this was assessed as a potential confounding influence.

Assessment of dietary intake and supplements

The SQFFQ (CSIRO, Adelaide, Australia) reported the frequency of consumption of 212 individual foods, mixed foods and beverages over the preceding month. Reported supplement intake (brand, dose, and frequency) was converted into daily folic acid intake (microgram/day) using dosage information provided on the packaging.

Calculation of Dietary Folate Equivalent units (DFE)

Folate measures were converted to DFEs to take into account the increased absorption and bioavailability of 'folic acid' from vitamin supplements compared with 'folate' derived from food (12). One microgram (μg) DFE = $1 \mu\text{g}$ of folate derived from food = $0.6 \mu\text{g}$ of folic acid from supplements and fortified foods consumed with food = $0.5 \mu\text{g}$ of folic acid from supplements taken on an empty stomach. The Recommended Daily Allowance (RDA) for folate during pregnancy in the USA (and in Australia where this population was derived) is $600 \mu\text{g}$ of DFEs (13). To reduce the risk of neural tube birth defects pregnant women are advised to consume $400 \mu\text{g}$ of DFEs of folic acid per day from dietary supplements and/or fortified foods in addition to food folate from a varied diet (13). A tolerable upper level (UL) of intake of folic acid from supplements for adults has been set at $1000 \mu\text{g}$ of DFEs/day (13).

Assessment of serum folate in mothers and infants

Maternal blood was collected in the third trimester, and cord blood was collected at delivery. Folate in serum was measured using a competitive immunoassay (Immulite 2000; Siemens Medical Solutions Diagnostics, Flanders, NJ, USA).

Assessment of allergic outcomes

At 12 months of age, the main clinical outcome measures were eczema, food allergy, and allergic sensitization. A child was classified as having 'allergic disease' if he/she had a physician diagnosis of IgE-mediated food allergy, eczema, or asthma. A diagnosis of eczema was made in infants with typical skin lesions (14). IgE-mediated food allergy was defined as a history of immediate symptoms following contact and/or ingestion and a positive SPT to the implicated food. A diagnosis of asthma was based on a history of recurrent wheeze (>2 episodes of wheezing) that was demonstrated to be responsive to bronchodilator medications, but the limitations are recognized at this age.

Allergen skin prick testing

Allergic sensitization was assessed by SPT at 1 year of age using common allergen extracts (milk, peanut, house dust mite, cat, grass, mold; Hollister-Stier Laboratories, Spokane, WA, USA), and whole egg. A wheal diameter of ≥ 2 mm was considered positive.

Assessment of confounding factors for folate status and the development of infant allergy

We collected detailed information about maternal factors that may influence folate status or risk of infant allergic disease, including maternal age, maternal allergic disease (and sensitization), previous pregnancies, socioeconomic status, and education level. Other information on postnatal exposures for the infants including daycare attendance, infection

history, postnatal dietary intervention, pet keeping, breast-feeding, and infant dietary patterns was also collected.

Statistical methods

All statistical analyses were performed using SPSS software (version 18 for Macintosh, SPSS Inc., Chicago, IL, USA). Folate data were not generally normally distributed and were expressed as median and interquartile range (IQR). Some folate data (serum folate levels and food frequency folate measures) were normalized with logarithmic transformation. Differences between groups and between related samples were determined by appropriate tests for nonparametric lognormal according to the data distribution. Associations between normally and lognormally distributed variables were evaluated in linear models (Pearson's correlation and linear regression).

Logistic regression was used to evaluate the effect of folate on binary allergic outcomes after adjusting for caloric intake or potential confounder variables (including maternal allergy, postnatal diet/feeding practices, exposure to pets, childcare, or older siblings). Folate variables were also categorized (to dummy variables based on tertiles) to determine effects of folate amounts on allergic outcomes compared with the defined reference category. Tertiles were assigned a consecutive integer to assess the trends of association between folate status and clinical outcome in logistic regression models.

Because of the exploratory nature of this study, we did not wish to exclude any important relationships by using stringent correction factors for multiple analyses. Accordingly, our findings have been interpreted conservatively.

Results

Population characteristics

Of the 628 women recruited, 594 had at least one measure of folate status (questionnaire or serum level) in pregnancy and 484 infants were assessed at 1 year of age (Table 1). One in three infants developed allergy, predominately eczema (35.4%) and food allergy (18.5%). Twenty-five percent were sensitized, mainly to food allergens (22.8% of infants tested). Because of the focus on allergy in the primary study, sensitized allergic mothers were over represented (70.6%) and as expected, their infants were more likely to develop allergic disease than those of nonallergic mothers ($n = 457$; OR = 1.65; 95% CI 1.07, 2.60; $P = 0.024$).

Folate intake in pregnancy (from diet and supplements)

Four hundred and seventy women completed the SQFFQ and supplied data on vitamin supplements taken in the third trimester. The median intake of folate, as food and supplement, was 992.8 μg DFE/day (IQR; 612.5–1523.4), indicating that <25% of these women did not reach the recommended daily intakes (13) of 600 μg DFE/day. Women consumed more folate from folic acid supplements than from folate-rich food (Table 2). Seventy-six percent ($n = 472$) were taking supplements containing folic acid, and the median dose of

Table 1 Population characteristics of the mothers and infants in the study

	Number analyzed	Mean $\pm$ SE or n (%)
Mothers at recruitment	628	
Age, mean years $\pm$ SE	624	32.9 $\pm$ 0.2
Gestation at recruitment, mean weeks $\pm$ SE	623	36.5 $\pm$ 0.08
History of allergic disease, n (%)	592	484 (81.8)
Asthma, n (%)	592	242 (40.8)
Hayfever, n (%)	592	372 (62.8)
Eczema, n (%)	591	223 (37.7)
Food allergy, n (%)	414	108 (18.5)
Skin prick test (SPT) positive, n (%)	615	458 (74.5)
Allergic disease and SPT positive, n (%)	588	415 (70.6)
Infants at 1 year	484	
Allergy disease, n (%)	484	172 (35.5)
Asthma, n (%)	484	0 (0)
Eczema, n (%)	477	169 (35.4)
Food allergy, n (%)	406	75 (18.5)
Infant SPT positive, n (%)	468	121 (25.9)
Egg SPT positive, n (%)		93 (20.0)
Peanut SPT positive, n (%)		43 (9.4)
Milk SPT positive, n (%)		17 (3.6)
HDM SPT positive, n (%)		20 (4.3)
Cat SPT positive, n (%)		16 (3.4)
Grasses SPT positive, n (%)		6 (1.3)

Table 2 Folate status of mothers during pregnancy and infants at birth

	Number	Median $\pm$ IQR
Maternal folate intake		
From food only, mg of DFE/day	476	277.9* (217.4–341.4)
From supplements only, mg DFE/day	617	668.0* (95.4–1210.8)
Combined food and supplements, mg of DFE/day	473	992.8 (612.5–1523.4)
Maternal and infant serum folate		
Maternal serum folate, nmol/l	435 [†]	37.2 [‡] (25.6–50.5)
Cord serum folate, nmol/l	285 [†]	63.2 [‡] (46.8–79.1)

DFE, dietary folate equivalent units; IQR, interquartile range.

*Folate intake was significantly higher from supplements compared with dietary food ($P < 0.001$).

[†]Characteristics of mothers and infants with samples did not differ from those in the total population shown in Table 1.

[‡]Serum folate was significantly higher in cord blood compared with maternal blood ($P < 0.001$).

synthetic folic acid they consumed was 400 μg /day (IQR; 343.4–800.0), equivalent to 668 μg of DFE/day (IQR 573.5–1336.0). Thirty-six percent ($n = 173$) of these women

exceeded the recommended tolerable upper limit of 1000 µg of DFEs from supplements/day. There were no major differences in the background dietary patterns of the women taking supplements.

There were no differences in folate intake between 'atopic' (sensitized) and nonatopic women ($P = 0.96$). There was also no difference in the overall folate intake, calculated as DFE units in women with a history of allergic 'disease' ($P = 0.64$). However, these women consumed more folic acid in vitamin supplements ($P = 0.013$) and less folate-rich food (total folate, $P = 0.014$; free folate, $P = 0.008$) than nonallergic mothers. Women with a tertiary education (79.6%) consumed more folate ($P = 0.002$), predominately as folic acid supplement ($P = 0.022$), but were not more likely to have a history of allergic disease ($P = 0.310$).

Folate levels in maternal and cord blood

Maternal serum folate levels

The median serum folate level during the third trimester was 37.2 nmol/l ($n = 435$; Table 2). This is in the upper range of the international reference range of 3–47 nmol/l in the third trimester (15). Serum folate was directly proportional to overall dietary folate intake (DFE) ($r = 0.490$, $P < 0.001$), and in a linear regression model, both folate ingested in food (DFE/day) ($\beta = 0.209$; 95% CI 0.051, 0.368; $P = 0.010$) and as a folic acid supplement (DFE/day) ($\beta = 0.312$; 95% CI 0.219, 0.405; $P < 0.001$) contributed significantly to maternal folate levels. There were no differences in folate levels of women with allergic disease and nonallergic women ($P = 0.26$).

Cord blood folate levels

The median level of folate in cord blood was 63.2 nmol/l. Neonatal folate was directly proportional to maternal folate ($r = 0.472$, $P < 0.001$), although cord blood folate was significantly higher than maternal levels ($P < 0.001$) (Fig. 1, Table 2). This suggests active or preferential fetal uptake of folate, and this (i.e., cord serum folate minus maternal serum

folate) was inversely proportional to maternal folate levels ($r = -0.329$, $P < 0.001$). Achieving maternal levels above 78.46 nmol/l did not result in further increase in cord blood levels, suggesting a saturable level of folate for the fetus.

Folic acid supplementation in pregnancy was a major determinant of neonatal folate levels ($P < 0.001$). In a linear regression model, folic acid consumed in vitamin supplements contributed more to neonatal folate status (B 0.205, 95% CI 0.032, 0.214; $P = 0.008$) than did folate-rich food (B 0.123, 95% CI -0.028, 0.274; $P = 0.109$).

Although there were no differences in *maternal* serum folate levels in allergic women, *cord blood* folate levels were higher if mothers had a history of allergic disease ($P = 0.013$). The reasons for this are not known but may relate to the higher use of supplements in the allergic women (and this was allowed for in the subsequent analyses).

Association between maternal folate status and infant allergic status at 1 year

First, we assessed the level of folate derived directly from the background diet. Although allergic women ate less folate-rich food in pregnancy, this was not associated with the development of allergies in infants (data not shown). There were no differences in the maternal dietary folate intakes of infants with eczema (Fig. 1) or any other allergic outcomes.

Second, we investigated whether infants of mothers consuming higher amounts of folic acid/day from vitamin supplements were more likely to develop allergy, allowing for any confounding effect of maternal allergy status (i.e., genetic differences in allergic risk). Infants who developed eczema had higher maternal folate supplement intake (as a continuous variable) than that of unaffected infants (Fig. 1, $P = 0.017$), and this remained significant after adjusting for maternal allergy. The amount of folic acid taken per day as a supplement was also categorized into tertiles <200, 200–500 and >500 µg folic acid/day. There was a significant association between the amount of folic acid taken as a supplement and the number of infants diagnosed with eczema at 1 year

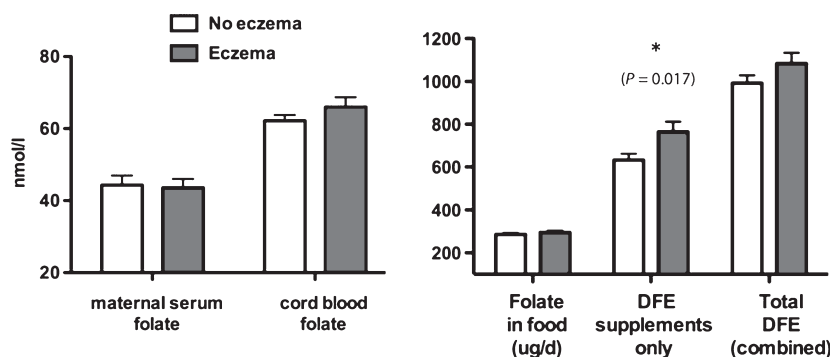


Figure 1 Comparison of *in utero* folate status in infants who developed subsequent eczema (shaded bars) compared with those who did not, including maternal and cord blood serum levels (nmol/l), folate derived from foods (µg/day based on FFQ) and dietary folate

equivalents (FFE) derived from supplements or from both diet and supplements (see Methods for calculations). Levels are shown as means and standard errors, and significance levels are based on *t*-tests of log-transformed data.

Table 3 Association between folate taken as vitamin supplements in pregnancy and allergic outcomes in 484 infants at 1 year of age

	Tertile 1 <200 mg/day	Reference [†] group	Tertile 2 200–499 mg/day	OR [‡] (95% CI)	Tertile 3 >500 mg/day	OR [‡] (95% CI)
Any allergic disease, <i>n</i> (%)	49 (30.6)	–	68 (37.6)	1.3 (0.8–2.1)	55 (39.9)	1.4 (0.9–2.4)
Sensitized, <i>n</i> (%)	34 (21.9)	–	49 (28.3)	1.3 (0.8–2.3)	36 (26.9)	1.2 (0.7–2.1)
Recurrent wheeze, <i>n</i> (%)	20 (12.6)	–	20 (11.2)	0.8 (0.4–1.7)	19 (14.0)	1.1 (0.6–2.3)
Eczema, <i>n</i> (%)	44 (27.3)	–	69 (39.0)*	1.5 (1.0–2.5)	55 (41.0)*	1.7 (1.0–2.8) [§]
Food reactions <i>n</i> (%)	21 (14.9)	–	33 (23.2)	1.7 (0.9–3.3)	21 (17.5)	1.2 (0.6–2.3)
IgE-mediated food allergy, <i>n</i> (%)	16 (10.1)	–	25 (14.1)	1.4 (0.7–3.0)	16 (11.9)	1.1 (0.5–2.4)
Sensitized to food allergens [§] , <i>n</i> (%)	31 (20.0)	–	44 (25.4)	1.3 (0.7–2.3)	30 (22.4)	1.1 (0.6–2.0)

*Indicates significant difference across the tertiles, Pearson's chi-square $P = 0.024$.

[†]Tertile 1: was used as reference group in regression.

[‡]Adjusted odds ratio and 95% confidence intervals: compared with reference group; adjusted for maternal allergy and infant postnatal diet (pets, siblings, and day care did not change the relationships and were not included).

[§]Indicates significant difference in tertile risk relative to reference group.

*Egg, peanut, and milk sensitization.

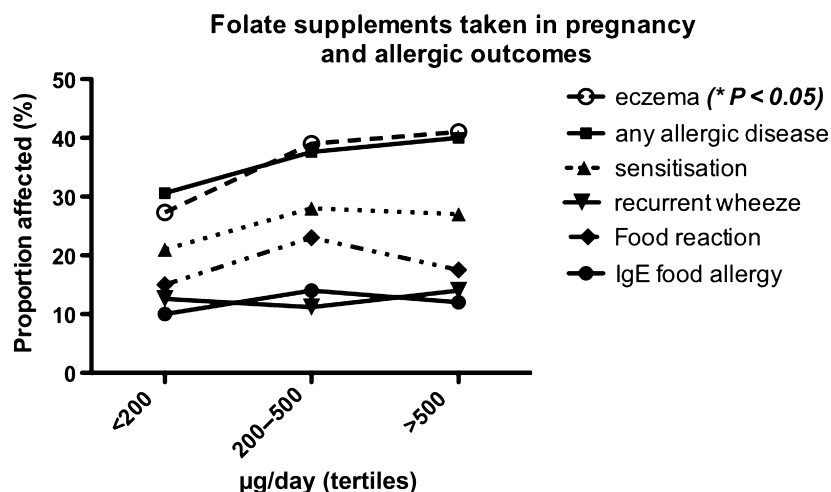


Figure 2 Comparison of infant allergic outcomes at 1 year of age according to maternal folic acid taken per day as a supplement in pregnancy. Groups are compared by tertiles: infants in tertile 1

(<200 µg/day), tertile 2 (200–499 µg/day), and tertile 3 (>500 µg folic acid/day). $P < 0.05$ is considered statistically significant.

of age (chi-square test, $P = 0.024$) (Table 3, Fig. 2). In a binary logistic regression model, infants whose mothers consumed more folate as supplements, i.e., in tertile 2 (200–499 µg/day; OR = 1.70 [1.07, 2.70]; $P = 0.012$) and tertile 3 (>500 µg/day; OR = 1.85 [1.14, 3.02]; $P = 0.013$) were more likely to develop eczema compared with those in tertile 1 with lower intake (<200 µg folic acid/day). After adjusting for maternal allergy status and other potential confounders, only the higher levels of folate in tertile 3 remained a significant predictor of eczema (OR = 1.30 [95% CI 1.01, 1.67]; $P = 0.040$). The analysis was repeated separately in allergic and nonallergic mothers to further allow for effects of genetic predisposition and higher supplement usage in allergic women. When sensitized allergic mothers ($n = 311$) were examined separately, higher levels of folate also predicted

eczema development. Compared with tertile 1 (<200 µg folic acid/day), those exposed to higher folate supplement were nearly twice as likely to develop eczema (tertile 2: 200–500 µg/day; OR = 1.88, 95% CI 1.06, 3.33; $P = 0.034$ and tertile 3: >500 µg/day; OR = 1.73, 95% CI 0.948, 3.16; $P = 0.074$). This comparison was not significant in the 138 (low risk) infants of nonallergic mothers. There were no other significant relationships between folic acid supplement intake in pregnancy and allergic outcomes, although there were non-significant trends for less total allergic disease and sensitization to be lower in children whose mothers consumed <200 µg folic acid/day in pregnancy (as seen on Fig. 2).

Finally, maternal serum folate levels were not associated with any infant outcomes at 1 year, including separate analysis of allergic and nonallergic mothers.

Table 4 Association between cord blood folate (nmol/l) and allergic outcomes in 229 infants at 1 year of age

	Tertiles of serum folate in neonates (nmol/l)					
	Tertile 1 <50.3 nmol/l	OR [†] (95% CI)	Tertile 2 50.3–75.1 nmol/l	Reference [‡] group	Tertile 3 >75.1 nmol/l	OR [†] (95% CI)
			Reference = 1			
Any allergic disease, <i>n</i> (%)	27 (33.3)	1.4 (0.7–2.9)	17 (23.6)	–	28 (36.8)	1.8 (0.9–3.6)
Sensitized, <i>n</i> (%)	18 (25.7)*	2.7 (1.1–7.0) [§]	8 (11.0)*	–	22 (30.1)*	3.3 (1.3–8.0) [§]
Recurrent wheeze, <i>n</i> (%)	11 (14.3)	0.8 (0.3–2.1)	8 (12.3)	–	8 (11.9)	0.9 (0.3–2.4)
Eczema, <i>n</i> (%)	26 (32.1)	0.9 (0.3–2.4)	21 (29.6)	–	24 (32.4)	0.8 (0.3–2.2)
Food allergy, <i>n</i> (%)	12 (16.9)	1.5 (0.5–4.4)	6 (10.3)	–	15 (24.2)	2.3 (0.9–6.5)
IgE-mediated food allergy, <i>n</i> (%)	9 (11.4)	1.7 (0.5–5.6)	4 (5.6)	–	12 (16.2)	2.6 (0.9–8.1)
Sensitized to food allergens [§] <i>n</i> (%)	17 (24.3)	2.2 (0.9–5.6)	9 (12.3)	–	19 (26.0)	1.1 (0.5–2.4)

*Indicates significant difference across the tertiles, Pearson's chi-square $P = 0.014$.

[†]Adjusted odds ratio and 95% confidence intervals: compared with reference group; adjusted for maternal allergy and infant postnatal diet (pets, siblings, and day care did not change the relationships and were not included).

[‡]Tertile 2: was used as reference group in regression.

[§]Indicates significant difference in tertile risk relative to reference group.

[¶]Egg, peanut, and milk sensitization.

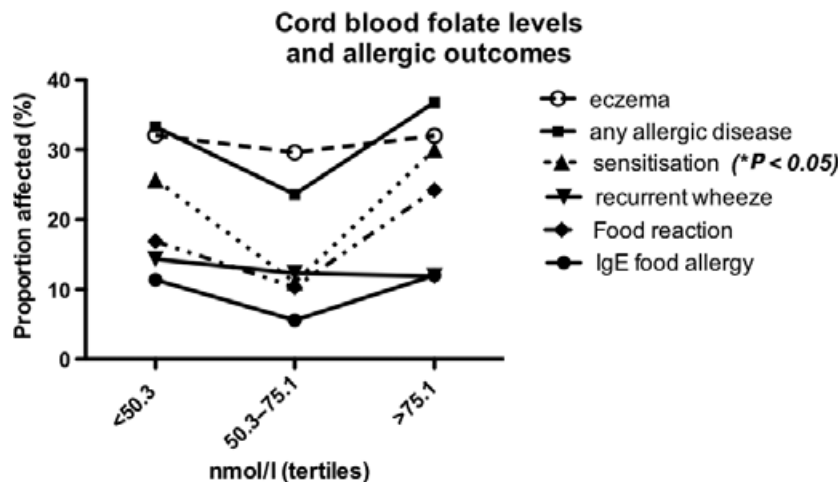


Figure 3 Comparison of infant allergic outcomes at 1 year of age according to fetal serum folate levels (detected in cord blood at birth). Groups are compared by tertiles: infants in tertile 1 (<50.3 nmol/l), tertile 2 (50.3–75.1 nmol/l), and tertile 3 (75.1 nmol/l). $P < 0.05$ is considered statistically significant.

Association between cord blood folate and infant allergic outcomes at 1 year

There were no significant differences in cord blood folate levels in children who developed eczema (Fig. 1) or other allergic symptoms, and in a logistic regression model, cord blood folate levels did not predict any infant allergic outcomes. However, when cord blood folate levels were categorized into tertiles, infants in the middle tertile appeared less likely to become sensitized at 1 year of age (Table 4, Fig. 3). Specifically, infants with cord blood folate levels > 75.1 nmol/l were nearly four times more likely (OR = 3.76; 95% CI 1.49, 9.51; $P = 0.005$) to be sensitized to at least one allergen compared to infants with cord blood folate levels between 50.3 and 75.1 nmol/l, and this remained significant after

adjusting for maternal allergy and sensitization (OR = 3.59; 95% CI 1.40, 9.20; $P = 0.008$). These infants were also more likely to be sensitized to food allergens, (egg, milk, and peanut, OR = 2.64; 95% CI 1.07, 6.52; $P = 0.035$) and have associated IgE-mediated symptoms of food allergy (OR = 2.77; 95% CI 0.99, 7.71; $P = 0.052$).

Similarly, infants with cord blood folate levels in the lower tertile (with cord folate level below 50.3 nmol/l) were three times more likely to be sensitized in the first year of life compared with infants in the middle tertile (OR = 2.96; 95% CI 1.16, 7.57; $P = 0.024$; adjusted for maternal allergy and sensitization; OR = 3.02; 95% CI 1.16, 7.87; $P = 0.024$).

Exposure to pets, childcare, or siblings did not contribute to or protect the infants from developing allergic disease and variables for these were not included in any of the models.

Discussion

In this study, the most common manifestation of allergic disease in infants, eczema, was not associated with any significant differences in maternal or cord blood serum folate levels. There were also no differences in the amounts of folate that mothers reported ingesting in their background diets. The only significant difference was that mothers of children with eczema appeared to consume more folate derived from supplements. However, this was only assessed in the third trimester and was not associated with any other allergic outcomes assessed at 1 year, including sensitization. In this population, the most significant source of folate intake in pregnancy was from specific folic acid supplements, and women taking more than 500 µg/day in this form (upper tertile) were at the highest risk of delivering an allergic child, even after adjusting for maternal allergic status. While the finding lends some support for epidemiological associations between maternal folate supplements in pregnancy and other allergic outcomes such as childhood asthma (6, 8) and wheezing (5), we did not see any effect on sensitization or wheezing *per se*. Although wheeze in the first year of life is nonspecific, it is also notable that another more recent study (the KOALA birth cohort) found no association between maternal supplementation and wheezing or asthma up to age 6–7 years, instead with a weak protective relationship between maternal folate levels and asthma (16). Consistent with our findings, eczema was also increased in the KOALA study when the mother had used folic acid during pregnancy (OR = 1.34; 95% CI [1.02–1.75]), although this lost statistical significance after adjusting for confounders (OR = 1.27; 95% CI 0.96–1.66) (16).

We have also provided novel data exploring how reported maternal folate intakes influenced fetal folate levels and how these levels were related to allergic disease. Interestingly, we did not see a linear relationship between fetal folate levels and allergic outcomes. Rather there appeared to be a 'U'-shaped relationship such that infants with higher cord blood levels (upper tertile of > 75 nmol/l) were at the greatest risk of allergic sensitization. Although this was consistent with the higher risk of allergy in those with higher reported maternal supplementation, we also saw an increased risk of allergic sensitization in those with 'relative deficient' cord blood levels (lower tertile; < 25 nmol/l). This suggests that there may be an 'optimal range' for folate levels in this context (middle tertile; 50–75 nmol/l for this population). At this stage, the significance of this is unclear, particularly, in relation to the contrasting (more linear) relationship with reported maternal supplementation. It is possible that these differences reflect the complex relationship between maternal serum and cord serum folate levels. Fetal levels were much higher (suggesting preferential fetal uptake), and while there was some correlation, this was < 0.5. This was a likely reflection of an apparent saturable limit, i.e., as maternal levels increased, proportional fetal uptake reduced. This is also likely to explain the discordant relationship between allergic outcomes that were related to cord blood levels but not to maternal serum levels. It could be argued that cord blood levels are a

more accurate measure of direct fetal exposure (than either 'reported' maternal supplement intake or maternal serum) and could therefore be of more relevance in this context.

Although there was a wide range of folate intakes in this population, the daily intake of 75% of the women was greater than the recommended dose of 600 µg DFE/day (13) mostly derived from vitamin supplements. Some of these women exceeded the tolerable UL of intake of synthetic folic acid of 1000 µg DFE/day. As indicated above, our findings also suggest that achieving higher maternal blood levels (above 78 nmol/l) through increased supplementation did not result in a further increase in cord blood concentrations. However, while there have been safety concerns relating to the presence of metabolically unaltered folic acid in the circulation, recent information suggests that unmetabolized folate does not accumulate substantially in cord blood (17).

The strengths of this study are that it comprised a well-characterized cohort with detailed allergic phenotypes of both mothers and their infants, based on not only clinical history but also confirmed by allergy SPT. We had biological measures, as well as food frequency questionnaire data, providing several different measures of folate status. On the other hand, there are a number of acknowledged weaknesses of our study. The population is over represented with allergic women and women with a tertiary education. However, it could be also argued that any effect of folate is most relevant on this population, already at recognized higher risk of having an allergic infant. Notably, we did demonstrate a separate effect of folate on allergic women who consumed higher amounts of folate as supplements. Finally, we only examined folate intake in the third trimester. It is not known at what stage of pregnancy folate status may be most important for allergic outcomes. Notably, Whitrow and colleagues (6) examined nutritional status at multiple time-points and found that the effects on allergic risk were only significant in the third trimester, well after the critical time for protection against neural tube defects. If this is borne out in further studies, there may be a case for more specific targeting of synthetic folate (folic acid) supplementation preconception and first trimester (as is currently the case) but perhaps not beyond. However, newer studies have found no significant relationships between folate use in early or late pregnancy (or preconception) and asthma and allergy risk (16).

Based on these conflicting findings (5, 6, 8, 16), it is clearly premature to consider any change in practice which remains important to prevent neural tube defects. Together with these other studies, our findings strengthen the need to explore the potential mechanisms of interaction between *in utero* folate exposure and the developing immune system in humans. This needs to include exploration of the potential epigenetic effects, which may change the developmental patterns of gene expression, as suggested in animal studies (4). There are likely to be a number challenges in this process including complex differences in the effects of folate on methylation in different tissues types and complex interactions with other dietary and environmental factors. This reinforces the need to define the early-life exposures that are driving the rise in disease suscep-

tibility, as these may provide valuable avenues for disease prevention.

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References

- Hanson M, Gluckman P. Developmental origins of noncommunicable disease: population and public health implications. *Am J Clin Nutr* 2011; [Epub ahead of Print].
- West CE, Videky D, Prescott SL. Role of diet in the development of immune tolerance in the context of allergic disease. *Curr Opin Pediatr* 2010;**22**:635–641.
- Bower C, D'Antoine H, Stanley FJ. Neural tube defects in Australia: trends in encephaloceles and other neural tube defects before and after promotion of folic acid supplementation and voluntary food fortification. *Birth Defects Res A Clin Mol Teratol* 2009;**85**:269–273.
- Hollingsworth JW, Maruoka S, Boon K, Garantzios S, Li Z, Tomfohr J et al. In utero supplementation with methyl donors enhances allergic airway disease in mice. *J Clin Invest* 2008;**118**:3462–3469.
- Håberg SE, London SJ, Stigum H, Nafstad P, Nystad W. Folic acid supplements in pregnancy and early childhood respiratory health. *Arch Dis Child* 2009;**94**:180–184.
- Whitrow MJ, Moore VM, Rumbold AR, Davies MJ. Effect of supplemental folic acid in pregnancy on childhood asthma: a prospective birth cohort study. *Am J Epidemiol* 2009;**170**:1486–1493.
- Ownby DR. Has mandatory folic acid supplementation of foods increased the risk of asthma and allergic disease? *J Allergy Clin Immunol* 2009;**123**:1260–1261.
- Håberg SE, London SJ, Nafstad P, Nilsen RM, Ueland PM, Vollset SE et al. Maternal folate levels in pregnancy and asthma in children at age 3 years. *J Allergy Clin Immunol* 2011;**127**:262–263.
- Meldrum SJ, D'Vaz N, Dunstan JA, Mori T, Prescott SL. The Infant Fish Oil Supplementation Study (IFOS): design and research protocol of a double-blind, randomized controlled n-3 LCPUFA intervention trial in term infants. *Contemp Clin Trials* 2011;**32**:771–778.
- Krauss-Etschmann S, Shadid R, Campoy C, Hoster E, Demmelmair H, Jiménez M et al. Nutrition and Health Lifestyle (NUHEAL) Study Group. Effects of fish-oil and folate supplementation of pregnant women on maternal and fetal plasma concentrations of docosahexaenoic acid and eicosapentaenoic acid: a European randomized multicenter trial. *Am J Clin Nutr* 2007;**85**:1392–1400.
- Crowe FL, Skeaff CM, McMahon A, Williams SM, Green TJ. Lowering plasma homocysteine concentrations of older men and women with folate, vitamin B-12, and vitamin B-6 does not affect the proportion of (n-3) long chain polyunsaturated fatty acids in plasma phosphatidylcholine. *J Nutr* 2008;**138**:551–555.
- Sutor CW, Bailey LB. Dietary folate equivalents: interpretation and application. *J Am Diet Assoc* 2000;**100**:88–94.
- Institute of Medicine FaNB, editor. Dietary reference intakes: thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline. Washington, DC: National Academy Press, 1998.
- Consensus Report of the European Task Force on Atopic Dermatitis. Severity scoring of atopic dermatitis: the SCORAD index. *Dermatology* 1993;**186**:23–31.
- Abbassi-Ghanavati M, Greer LG, Cunningham FG. Pregnancy and laboratory studies: a reference table for clinicians. *Obstet Gynecol* 2009;**114**:1326–1331.
- Magdelijns FJH, Mommers M, Penders J, Smits L, Thijs C. Folic acid use in pregnancy and the development of atopy, asthma, and lung function in childhood. *Pediatrics* 2011;**128**:e135–e144.
- Obeid R, Kasoha M, Kirsch SH, Munz W, Herrmann W. Concentrations of unmetabolized folic acid and primary folate forms in pregnant women at delivery and in umbilical cord blood. *Am J Clin Nutr* 2010;**92**:1416–1422.

Author contribution

Janet A. Dunstan performed the analysis and prepared the manuscript. Susan L. Prescott designed, coordinated and supervised the study and prepared the manuscript. Christina West assisted in the analysis and provided intellectual input.

Suzi McCarthy, Jessica Metcalfe, Nina D'Vaz, Suzanne Meldrum collected and prepared the data used in the study including assistance with clinical follow-up visits. Wendy H. Oddy and Meri K. Tulic provided intellectual input.

Conflicts of interest

None of the aforementioned authors has any 'Conflicts of interest' relevant to the work of this publication.