

Maternal B vitamin intake during pregnancy and wheeze and eczema in Japanese infants aged 16–24 months: The Osaka Maternal and Child Health Study

Miyake Y, Sasaki S, Tanaka K, Hirota Y. Maternal B vitamin intake during pregnancy and wheeze and eczema in Japanese infants aged 16–24 months: The Osaka Maternal and Child Health Study. *Pediatr Allergy Immunol* 2010.
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It is uncertain whether B group vitamins are risk or preventive factors for allergic disorders. We prospectively investigated the association between maternal intake of folate and vitamins B₁₂, B₆, and B₂ during pregnancy and the risk of wheeze and eczema in the infants aged 16–24 months. Subjects were 763 Japanese mother–child pairs. Data on maternal intake during pregnancy were assessed with a diet history questionnaire (DHQ). Symptoms of wheeze and eczema were based on criteria of the International Study of Asthma and Allergies in Childhood. Among 763 infants, 169 (22.1%) and 142 (18.6%) had symptoms of wheeze and eczema, respectively. There were no evident relationships between maternal consumption of folate, vitamin B₁₂, vitamin B₆, and vitamin B₂ during pregnancy and the risk of wheeze or eczema in the offspring after adjustment for maternal age, gestation at baseline, residential municipality at baseline, family income, maternal and paternal education, maternal and paternal history of asthma, atopic eczema, and allergic rhinitis, changes in maternal diet in the previous 1 month, season when data at baseline were collected, maternal smoking during pregnancy, baby's older siblings, baby's sex, baby's birth weight, household smoking in the same room as the infant, breastfeeding duration, age at which solid foods were introduced, age of infant at the third survey, and maternal intake of docosahexaenoic acid, n-6 polyunsaturated fatty acids, vitamin D, calcium, vitamin E, and β -carotene during pregnancy. Further investigation is warranted to draw conclusions as to the question of whether maternal B vitamin intake during pregnancy is related to the risk of childhood allergic disorders.

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Key words: B vitamins; cohort study; eczema; Japanese infants; maternal diet; pregnancy; wheeze

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Accepted 24 April 2010

It is uncertain whether B group vitamins such as folate and vitamin B₁₂ are risk or preventive factors for allergic disorders. In a cross-sectional study among 8083 subjects 2 yr of age and older using the 2005–2006 NHANES dataset, a higher serum folate level was significantly associated with lower total IgE levels and a decreased prevalence of atopy and wheeze, but not doctor-diagnosed asthma (1). A cross-sectional study of

Danish adults aged 30–60 showed that homozygosity for the T allele of the C677T of the methylenetetrahydrofolate reductase (MTHFR) gene was significantly related to an increased prevalence of atopy and that higher intake of folate and vitamin B₂ was significantly associated with a reduced prevalence of atopy only in persons with the MTHFR TT genotype (2). However, neither dietary folate intake nor the

MTHFR C677T genotype was cross-sectionally related to a history of asthma or allergy among UK pregnant women (3).

With regard to epigenetics, folate metabolism is profoundly implicated in the DNA methylation pathway. The development of a polarized Th2 phenotype associated with atopy may result from altered DNA methylation at genes regulating Th differentiation (4). A cohort study in Norway found that folate supplements during weeks 0–12 of pregnancy were statistically significantly associated with a slightly increased risk of wheeze in the offspring between 6 and 18 months of age (5). In a previously cited UK study, neither maternal folate intake at 32 wk of pregnancy (excluding supplements) nor use of folate supplements at 18 and 32 wk of pregnancy were related to the risk of atopy in children aged 7–8 (3).

In view of the scarcity of epidemiological information regarding the relationship between intake of B vitamins and allergic disorders, we investigated the effects of maternal consumption of folate and vitamins B₁₂, B₆, and B₂ during pregnancy on the risk of wheeze and eczema in Japanese infants at 16–24 months of age using data from the Osaka Maternal and Child Health Study (OMCHS).

Methods

Study population

The OMCHS is a prospective cohort study. Details of the OMCHS have been described elsewhere (6, 7). We recruited pregnant women at baseline. Neyagawa City is one of the 43 municipalities in Osaka Prefecture, a metropolis in Japan. Of the 3639 eligible subjects in Neyagawa City, 627 women (17.2%) participated in the OMCHS between November 2001 and March 2003. In order to increase the sample size, pregnant women living in municipalities other than Neyagawa City were also recruited. Eight pregnant women who lived in municipalities adjacent to Neyagawa City and who had become aware of the OMCHS at an obstetric clinic before August 2002 decided by themselves to participate in the OMCHS. Also, there were 77 participants who received explanations of the OMCHS from public health nurses in six other municipalities from August 2002 to March 2003. From October 2002 to March 2003, 290 participants were recruited from a university hospital and three obstetric hospitals in three other municipalities.

Finally, a total of 1002 pregnant women between the 5th and 39th week of pregnancy

gave their fully informed consent in writing and completed the baseline survey. Of the 1002 women, 867 mother–child pairs participated in the second survey. The second survey was carried out from 2 to 9 months postpartum. Of the 867 mother–child pairs, 763 participated in the third survey from 16 to 24 months postpartum and were left for the present analysis. The protocol of the OMCHS was approved by the ethics committee of the Osaka City University School of Medicine.

Measurements

Baseline assessment of the OMCHS consisted of a set of two self-administered questionnaires. Also, a self-administered questionnaire was used in both the second and third survey. Participants mailed the answered questionnaires to the data management center at the time of each survey. Research technicians completed missing or illogical data by telephone interview.

One of the self-administered questionnaires at baseline was a semi-quantitative, comprehensive, diet history questionnaire (DHQ) that assesses dietary habits during the preceding month (8, 9). The DHQ is a 16-page structured questionnaire that asks about the consumption frequency and portion size of selected foods commonly consumed in Japan as well as general dietary behavior and usual cooking methods (8). Estimates of daily intake of foods (150 items in total), energy, and selected nutrients were calculated using an *ad hoc* computer algorithm for the DHQ (8, 9), based on the Standard Tables of Food Composition in Japan (10, 11). Because only a small number of participants used vitamin B complex (4.1%) and multivitamin (4.2%) supplements at least once a week at baseline, information on these dietary supplements was not considered in the present analysis. According to a validation study of 92 women of 31–69 yr of age, Pearson's correlation coefficients between the DHQ and 16-day weighed dietary records were 0.60 for folate, 0.50 for vitamin B₁₂, 0.66 for vitamin B₆, 0.52 for vitamin B₂, 0.44 for marine origin n-3 polyunsaturated fatty acids, 0.39 for n-6 polyunsaturated fatty acids, 0.54 for vitamin D, 0.56 for calcium, 0.39 for vitamin E, and 0.64 for β -carotene (Sasaki S, unpublished data, 2006). Energy-adjusted intake by the residual method was used for the analyses (12).

A second questionnaire at baseline asked about maternal age, gestation, family income, maternal and paternal education, maternal and paternal history of asthma, atopic eczema, and allergic rhinitis, and changes in diet in the

previous 1 month. A paternal or maternal history of asthma, atopic eczema, and allergic rhinitis was defined as positive if the respective parent had been treated with medications for any of these allergic disorders at some time prior to the start of the baseline survey.

A self-administered questionnaire in the second survey elicited information on the baby's sex, birth weight, and date of birth, number of baby's older siblings, maternal smoking during pregnancy, and smoking in the household. A self-administered questionnaire in the third survey included questions on breastfeeding duration in months, the age in months at which solid foods were introduced, and symptoms of wheeze and eczema based on the International Study of Asthma and Allergies in Childhood (ISAAC) phase-I questionnaire (13, 14). Wheeze was defined as present if the mother answered 'yes' to the question 'Has your child had wheezing or whistling in the chest in the last 12 months?'. For eczema, affirmative answers to the following three questions were required: 'Has your child ever had an itchy rash which was coming and going for at least 6 months?', 'Has your child had this itchy rash at any time in the last 12 months?' and 'Has this itchy rash at any time affected any of the following places: the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears, or eyes?'

Statistical analysis

Intake of dietary factors under study was categorized at quartile points based on the distribution in 763 subjects. Maternal age, gestation at baseline, residential municipality at baseline, family income, maternal and paternal education, maternal and paternal history of asthma, atopic eczema, and allergic rhinitis, changes in maternal diet in the previous 1 month, season when data at baseline were collected, maternal smoking during pregnancy, baby's older siblings, baby's sex, baby's birth weight, household smoking in the same room as the infant, breastfeeding duration, age at which solid foods were introduced, age of the infant at the third survey, and maternal intake of docosahexaenoic acid, n-6 polyunsaturated fatty acids, vitamin D, calcium, vitamin E, and β -carotene during pregnancy were *a priori* selected as potential confounding factors. Our previous findings using data from the OMCHS showed significant inverse associations between maternal intake of docosahexaenoic acid (15), vitamin D, calcium (16), and vitamin E (17) during pregnancy and the risk of wheeze in the infants aged 16–24 months. Maternal

consumption of vitamin D (16), vitamin E, and β -carotene (17) was inversely related to the risk of infantile eczema, whereas maternal n-6 polyunsaturated fatty acid intake (15) was positively associated with infantile eczema in our previous reports.

Logistic regression analysis was performed to estimate crude odds ratios (ORs) and their 95% confidence intervals (CIs) of wheeze and eczema according to the quartile of dietary factors under investigation, with the lowest quartile as the reference. Multiple logistic regression analysis was employed to adjust for potential confounding factors. Trend of association was assessed by a logistic regression model assigning consecutive integers (1 to 4) to the quartiles of the exposure variables. All statistical analyses were carried out using the SAS software package version 9.1 (SAS Institute, Inc., Cary, NC, USA).

Results

Among 763 infants, 169 (22.1%) and 142 (18.6%) had symptoms of wheeze and eczema based on the ISAAC criteria, respectively, in the third survey. About 75% of the infants had been breastfed for 6 months or longer (Table 1). Slight or substantial changes in diet in the previous 1 month were reported by 551 mothers because of nausea gravidarum ($n = 453$), maternal and fetal health ($n = 89$), and other reasons ($n = 9$). Compared with children without wheeze, those with wheeze were more likely to have mothers and fathers with a history of asthma and have older siblings. In comparison with children without eczema, those with eczema were more likely to have mothers with a history of atopic eczema and allergic rhinitis, have fathers with a history of atopic eczema, and have mothers who participated in the baseline survey in spring and summer. Maternal mean daily total energy consumption and energy-adjusted intake of folate, vitamin B₁₂, vitamin B₆, and vitamin B₂ during pregnancy were 7628.6 kJ, 284.0 μ g, 5.5 μ g, 1.0 mg, and 1.3 mg, respectively (Table 2).

Compared with maternal consumption of vitamin B₂ during pregnancy in the first quartile, such consumption in the fourth quartile was significantly related to a reduced risk of wheeze in the offspring by crude analysis (Table 3). However, the inverse relationship completely disappeared after adjustment for confounders under investigation. Maternal intake of folate, vitamin B₁₂, and vitamin B₆ during pregnancy was not materially associated with the risk of wheeze in the children in the multivariate model. No significant relationships were observed

Table 1. Distribution of selected characteristics of 763 parent–child pairs

Variable	n (%) or mean (s.d.)
Baseline characteristics	
Maternal age (yr)	30.0 (4.0)
Gestation (wk)	17.7 (6.7)
Residential municipality	
Neyagawa City	457 (59.9)
Other than Neyagawa City	306 (40.1)
Family income (% yen/yr)	
<4,000,000	211 (27.7)
4,000,000–5,999,999	313 (41.0)
≥6,000,000	239 (31.3)
Maternal education (% yr)	
<13	212 (27.8)
13–14	329 (43.1)
≥15	222 (29.1)
Paternal education (% yr)	
<13	289 (37.9)
13–14	127 (16.6)
≥15	347 (45.5)
Maternal history of asthma (%)	80 (10.5)
Maternal history of atopic eczema (%)	120 (15.7)
Maternal history of allergic rhinitis (%)	256 (33.6)
Paternal history of asthma (%)	64 (8.4)
Paternal history of atopic eczema (%)	72 (9.4)
Paternal history of allergic rhinitis (%)	143 (18.7)
Changes in diet in the previous 1 month (%)	
None	212 (27.8)
Slight	336 (44.0)
Substantial	215 (28.2)
Season when data were collected (%)	
Spring	241 (31.6)
Summer	127 (16.6)
Fall	167 (21.9)
Winter	228 (29.9)
Characteristics at the postnatal assessment	
Maternal smoking during pregnancy (%)	97 (12.7)
Baby's older siblings (% 1 or more)	381 (49.9)
Baby's sex (% male)	403 (52.8)
Baby's birth weight (g)	3071.0 (415.5)
Household smoking in same room as infant	195 (25.6)
Breastfeeding duration (% months)	
<6	189 (24.8)
≥6	574 (75.2)
Age at which solid foods were introduced (% months)	
<6	444 (58.2)
≥6	319 (41.8)
Age of infant at third survey (% months)	
16–19	263 (34.5)
20	370 (48.5)
21–24	130 (17.0)

between maternal consumption of folate, vitamin B₁₂, vitamin B₆, and vitamin B₂ during pregnancy and the risk of eczema in the offspring after adjustment for confounders.

Discussion

Folate plays a crucial role in nucleic acid and protein synthesis by supplying one-carbon units, and therefore inadequate folate significantly alters the immune response (18). Vitamins B₁₂,

Table 2. Distribution of daily intake of total energy and selected vitamins in 763 pregnant women at baseline*

Variable	Mean (s.d.)
Total energy (kJ)	7628.6 (1924.1)
Folate (μg)	284.0 (82.2)
Vitamin B ₁₂ (μg)	5.5 (2.6)
Vitamin B ₆ (mg)	1.0 (0.3)
Vitamin B ₂ (mg)	1.3 (0.3)
Docosahexaenoic acid (g)	0.30 (0.17)
n-6 Polyunsaturated fatty acids (g)	11.5 (2.7)
Vitamin D (μg)	6.2 (3.7)
Calcium (mg)	542.3 (170.7)
Vitamin E (mg)	7.8 (1.7)
β-Carotene (μg)	2620.4 (1653.0)

*Nutrient intake was adjusted for total energy intake using the residual method.

B₆, and B₂ are key cofactors for one-carbon metabolism. In the current study, there were no evident relationships between maternal consumption of folate, vitamin B₁₂, vitamin B₆, and vitamin B₂ during pregnancy and the risk of wheeze or eczema in the offspring. In this population, maternal B vitamin intake during pregnancy might not have the potential to influence fetal immune maturation. Japan has not yet mandated food fortification with folic acid. Maternal B vitamin intake in our population was similar to that in the US studies conducted before the folate fortification program (19). Compared with B vitamin intake in our population, much lower B vitamin intake during pregnancy might detrimentally affect antenatal immune development, whereas higher intake might have a beneficial effect. Information on genetic characteristics such as single nucleotide polymorphisms influencing one-carbon metabolism was not available in this study. Thus, future research that examines the interaction between such polymorphisms and B vitamin intake during pregnancy with regard to the risk of allergic disorders in the children would be of interest.

The current findings are not likely to support the hypothesis based on epigenetic mechanisms that *in utero* exposure to a diet rich in methyl donors could increase the risk of childhood allergic disorders. A laboratory study in mice showed that exposure *in utero* to a high-methyl donor diet, which consisted of folic acid, vitamin B₁₂, choline, L-methionine, zinc, betaine, cyanocobalamin, and genistein, enhanced cardinal features of allergic airway disease in the offspring through increased DNA methylation, including airway hyper-reactivity, higher concentrations of eosinophils and IL-13 in lung lavage fluid, and higher concentrations of serum IgE and that this predisposition appears to be partially transmitted

Table 3. Odds ratios (ORs) and 95% confidence intervals (CIs) of wheeze and eczema in 763 infants aged 16–24 months by quartiles of maternal intake of folate and vitamins B₁₂, B₆, and B₂ during pregnancy

Variable*	Wheeze			Eczema		
	No. cases	Crude OR (95% CI)	Adjusted OR (95% CI)†	No. cases	Crude OR (95% CI)	Adjusted OR (95% CI)†
Folate						
Q1 (206.8)	44	1.00	1.00	44	1.00	1.00
Q2 (255.1)	38	0.82 (0.50–1.34)	1.12 (0.65–1.95)	26	0.52 (0.30–0.89)	0.57 (0.31–1.01)
Q3 (291.2)	47	1.08 (0.68–1.74)	1.56 (0.89–2.75)	37	0.80 (0.49–1.30)	1.00 (0.57–1.79)
Q4 (370.6)	40	0.88 (0.54–1.43)	1.28 (0.65–2.50)	35	0.74 (0.45–1.22)	1.01 (0.51–2.00)
p for trend		0.89	0.27		0.49	0.57
Vitamin B ₁₂						
Q1 (3.3)	45	1.00	1.00	44	1.00	1.00
Q2 (4.6)	46	1.02 (0.64–1.64)	1.18 (0.69–2.01)	32	0.67 (0.40–1.11)	0.63 (0.35–1.11)
Q3 (5.8)	43	0.94 (0.58–1.51)	1.40 (0.77–2.57)	34	0.72 (0.43–1.18)	0.83 (0.44–1.55)
Q4 (7.8)	35	0.72 (0.44–1.19)	1.27 (0.59–2.73)	32	0.67 (0.40–1.11)	0.85 (0.39–1.84)
p for trend		0.19	0.42		0.15	0.77
Vitamin B ₆						
Q1 (0.7)	56	1.00	1.00	43	1.00	1.00
Q2 (0.9)	34	0.52 (0.32–0.84)	0.58 (0.33–1.001)	33	0.71 (0.43–1.18)	0.74 (0.42–1.32)
Q3 (1.0)	36	0.56 (0.34–0.89)	0.66 (0.37–1.17)	32	0.69 (0.41–1.14)	0.81 (0.44–1.48)
Q4 (1.2)	43	0.70 (0.44–1.10)	0.92 (0.47–1.77)	34	0.74 (0.45–1.22)	1.06 (0.52–2.15)
p for trend		0.14	0.85		0.24	0.86
Vitamin B ₂						
Q1 (1.0)	51	1.00	1.00	42	1.00	1.00
Q2 (1.2)	39	0.70 (0.43–1.12)	1.06 (0.61–1.84)	33	0.74 (0.44–1.22)	0.76 (0.42–1.35)
Q3 (1.4)	45	0.84 (0.53–1.34)	1.44 (0.81–2.57)	36	0.82 (0.50–1.35)	0.86 (0.47–1.59)
Q4 (1.7)	34	0.59 (0.36–0.96)	1.27 (0.64–2.52)	31	0.68 (0.41–1.14)	0.72 (0.34–1.49)
p for trend		0.08	0.32		0.20	0.49

*Quartile medians µg/day (quartile medians mg/day for vitamins B₆ and B₂) adjusted energy intake using the residual method are given in parentheses.

†Adjustment for maternal age, gestation at baseline, residential municipality at baseline, family income, maternal and paternal education, maternal and paternal history of asthma, atopic eczema, and allergic rhinitis, changes in maternal diet in the previous 1 month, season when data at baseline were collected, maternal smoking during pregnancy, baby's older siblings, baby's sex, baby's birth weight, household smoking in same room as infant, breastfeeding duration, age at which solid foods were introduced, age of infant at the third survey, and maternal intake of docosahexaenoic acid, n-6 polyunsaturated fatty acids, vitamin D, calcium, vitamin E, and β-carotene during pregnancy.

to subsequent generations (20). A positive relationship was observed between folate supplementation in the first trimester and the risk of wheeze in the infants in a Norwegian cohort study (5). Thus, too much dietary supplementation with methyl donors during a vulnerable period of fetal development might modify the heritable risk of allergic disorders in the offspring via epigenetic mechanisms.

The current study had methodological advantages. Study subjects were homogeneous with respect to having the same residential background. A variety of potential confounding factors were adjusted for. The prospective design was likely to reduce the possibility of recall bias. Definitions of wheeze and eczema were based on the ISAAC questions although validation tests of the ISAAC questions have not been performed for Japanese infants. The outcomes under investigation were ascertained for offspring at varying ages between 16 and 24 months. Moreover, wheeze and eczema are very different conditions, and wheeze in infancy is known not to be a good predictor of asthma over the longer term. These

disadvantages would have led to an underestimation of values in our results.

Other weaknesses in this study also should be borne in mind. Our DHQ could only approximate consumption, although the correlation between the DHQ and dietary records was reasonable, as described earlier. The consequence would have given rise to an underestimation of our results. Our DHQ was designed to assess recent dietary intake, i.e. for 1 month prior to completing the questionnaire. This disadvantage is likely to be alleviated after adjustment for the season when data were collected, however. Changes in diet in the past 1 month were controlled for because pregnant women are likely to change their diet for reasons such as nausea gravidarum. Dietary assessment was performed from the 5th to the 39th week of pregnancy. When our subjects were divided into two groups according to gestation at baseline, relationships between maternal B vitamin intake during pregnancy and the risk of wheeze and eczema in infants whose mothers participated in the baseline survey by the 17th week of gestation

(n = 401) were generally similar to those in infants whose mothers took part in the baseline survey after the 18th week of gestation (n = 362).

Of the 1002 participants at baseline, 239 mother-child pairs did not take part in the third survey. There were no material differences between the 239 non-participants and the 763 participants in the third survey with regard to distribution of maternal age, maternal and paternal history of asthma, atopic eczema, and allergic rhinitis, and maternal intake of total energy, folate, and vitamins B₁₂, B₆, and B₂. Compared with non-participants in the third survey, participants were less likely to report low family income and a low maternal and paternal educational level. Moreover, at baseline, the participation rate in Neyagawa City was only 17.2%. In other areas, the participation rate could not be calculated because the exact number of eligible subjects among the sources from which participants were recruited was not available. Thus, the mother-child pairs in this study were likely not representative of Japanese mother-child pairs in the general population. In fact, educational levels were higher in the mothers in our study than in the general population. According to the 2000 population census of Japan, the proportions of women aged 30–34 in Osaka Prefecture with years of education of < 13, 13–14, 15+, and unknown were 49.2, 32.3, 13.6, and 4.9%, respectively (21). The present population might have had a greater awareness about health than the general population.

To the best of our knowledge, there has been no birth cohort study on the relationship between maternal consumption of B vitamins during pregnancy and the risk of allergic disorders in the offspring. Our results suggest that maternal intake of folate, vitamin B₁₂, vitamin B₆, and vitamin B₂ during pregnancy was not measurably associated with the risk of wheeze or eczema in the offspring. Further investigation is warranted to draw conclusions as to the question of whether maternal B vitamin intake during pregnancy is related to the risk of childhood allergic disorders.

Acknowledgments

The authors would like to acknowledge the Neyagawa City Government, Hirakata City Government, Katano City Government, Shijonawate City Government, Kaizuka City Government, Takaishi City Government, Hannan City Government, Neyagawa City Medical Association, Hirakata City Medical Association, and the Kadoma City Medical Association for their valuable support.

This study was supported by a Grant-in-Aid (13770206, 16790351) for Scientific Research from the Ministry of

Education, Culture, Sports, Science, and Technology and Health and Labour Sciences Research Grants, Research on Allergic Disease and Immunology from the Ministry of Health, Labour, and Welfare, Japan.

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