Low Maternal Vitamin E Intake during Pregnancy Is Associated with Asthma in 5-Year-Old Children

Graham Devereux, Stephen W. Turner, Leone C. A. Craig, Geraldine McNeill, Sheelagh Martindale, Paul J. Harbour, Peter J. Helms, and Anthony Seaton

Departments of Environmental and Occupational Medicine and Child Health, University of Aberdeen, Aberdeen, United Kingdom

Rationale: **We have previously reported an association between reduced maternal vitamin E intake during pregnancy and wheezing in 2-yr-old children.**

Objectives: **To assess whether maternal nutrient intake during pregnancy is associated with asthma-related outcomes in children aged 5 yr.**

Methods: **A longitudinal cohort study of 1,861 children born to women recruited during pregnancy and followed up at 5 yr.**

Measurements: **Maternal nutrient status was assessed by a food frequency questionnaire and plasma levels. Respiratory and food frequency questionnaires were completed at 5 yr and children were invited for measurement of spirometry and skin-prick testing.**

Main Results: **Symptom and food frequency questionnaire data were available for 1,253 and 1,120 children, respectively; 700 children were skin prick tested, and FEV_1 was measured in 478 and exhaled nitric oxide in 167 children. In 5-yr-old children, maternal vitamin E intake during pregnancy was negatively associated with wheeze in previous year (odds ratio per intake quintile, 0.82; 95% confidence interval, 0.71–0.95), asthma ever (0.84, 0.72–0.98), asthma and wheeze in previous year (0.79, 0.65–0.95), and persistent wheezing (0.77, 0.63–0.93). Maternal plasma α-tocopherol during pregnancy was positively associated with post-bronchodilator FEV_1 at 5 yr, with a 7-ml (95% confidence interval, 0–14; $p = 0.04$) increase in FEV_1 per μg/ml α-tocopherol. Maternal zinc intake during pregnancy was negatively associated with asthma ever (0.83, 0.71–0.78) and active asthma (0.72, 0.59–0.89). There were no associations between children's nutrient intake and respiratory outcomes.**

Conclusion: **Maternal intake of foods containing vitamin E and zinc during pregnancy is associated with differences in the risks of developing childhood wheeze and asthma.**

Keywords: children; pregnancy; ventilatory function; vitamin E; zinc

In 1994, we hypothesized that the recent well-documented increase in asthma prevalence in affluent countries is, in part, a consequence of decreasing dietary antioxidant intake (1). In epidemiologic studies of children and adults, several groups have reported associations between asthma and reduced intake and blood levels of dietary nutrients such as antioxidant vitamins (vitamins C, E, and β-carotene) (2–5) and trace elements (selenium, zinc, copper, iron, manganese, and magnesium), some of which have antioxidant properties (6–11). However, supplementation with antioxidants (vitamins C and E) and trace elements (selenium, magnesium) has not been consistently associated with improved asthma outcomes (12–15). A possible explanation for the inconsistencies between epidemiologic and intervention studies is that dietary antioxidants and trace elements primarily influence the development of asthma during a critical time period early in life. Such a model does not preclude the possibility of minor effects later in life. If reduced antioxidant and trace element intakes during a critical period early in life increases the likelihood of asthma in later life, cross-sectional studies in adulthood would not be able to confirm or refute a role in disease initiation, and dietary supplementation in adults with established asthma is unlikely to be effective. This is an important issue and can only be answered by following cohorts from fetal life to disease expression.

We have established a cohort of children recruited *in utero* to test the hypothesis that maternal nutrient intake during pregnancy influences susceptibility to childhood asthma. In a previous report on this cohort, we found that 2-yr-old children whose mothers' vitamin E intake during pregnancy had been relatively low were more likely to wheeze in the absence of a cold (16). We also reported an unexpected adverse association between higher maternal vitamin C intake and wheeze in the 2-yr-old children but suggested that this could be due to confounding by "health consciousness," whereby mothers who were more conscious of their own and their children's health were both more likely to follow dietary advice to eat more fruit and more likely to notice and report minor wheezing episodes in their young children.

In this article, we describe the results of the 5-yr follow-up of the cohort. This was performed to determine whether the associations between wheeze in 2-yr-old children and maternal nutrient intake during pregnancy persist into later childhood when the asthma phenotype is more clearly established and the children can be more fully characterized. Some of the preliminary findings of this study have been previously reported in the form of an abstract (17).

METHODS

Study Subjects and Protocol

Full details of recruitment have been described previously (16). A total of 2,000 healthy pregnant women attending antenatal clinics, at a median of 12 wk gestation, were recruited over 19 mo during 1997 and 1999. There was no selection for asthma or atopic disease and, apart from expected slight biases, the recruited women were representative of the local obstetric population (16). At enrollment, an interviewer administered a questionnaire to the women, atopic status was ascertained by skin-prick testing, and a nonfasting venous blood sample was obtained. At 32 wk gestation, dietary intake over the preceding 3 mo was assessed using version 5.4 of the Scottish Collaborative Group Food Frequency Questionnaire (FFQ). In 40 women of childbearing age, the rank correlation coefficients for intakes of vitamin C, vitamin E, β-carotene, and zinc derived by this questionnaire and 4-d weighed records were 0.59 ($p < 0.001$), 0.52 ($p < 0.001$), 0.44 ($p < 0.01$), and 0.57 ($p < 0.001$) (18). At delivery, maternal and infant (umbilical cord blood) plasma were sampled.

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Correspondence and requests for reprints should be addressed to G. Devereux, M.D., Ph.D., Department of Environmental and Occupational Medicine, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZB, UK. E-mail: g.devereux@abdn.ac.uk

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Plasma Antioxidant Analysis

Plasma concentrations of vitamin E (α-tocopherol) and β-carotene were determined by normal phase high-performance liquid chromatography (19). Plasma ascorbate and zinc concentrations were measured using enzymatic colorimetric assays (20, 21).

Assessment of Children

Singletons born to the cohort of women were followed up at 5 yr. Six weeks before the fifth birthday of each child in the study, a questionnaire based on the ISAAC (International Study of Asthma and Allergies in Childhood) questionnaire (16, 22) was mailed to the child's family, followed by up to two reminders if needed. Wheeze was defined by an affirmative response to the question "Has your child had wheezing or whistling in the chest in the last 12 months?" Similar questions inquired about "ever wheezed" and "wheezed in the absence of a cold." Doctor-diagnosed asthma was defined by a positive response to the question "Has your child ever suffered from asthma which has been diagnosed by a doctor?" Similar questions inquired about breathlessness and doctor-diagnosed eczema.

Parents responding to the questionnaire were invited to complete an FFQ (version C1) to assess the dietary intake of the study child over the previous 3 mo. Version C1 is a 121-item semiquantitative FFQ based on the questionnaire used for the mothers in pregnancy (18) but modified for preschool children aged 3–5 yr by simplifying the response choices and changing the food list and portion sizes. In 74 children aged 3–5 yr recruited from local nurseries, the rank correlation coefficients for intakes of vitamin C, vitamin E, and zinc derived by this FFQ and 4-d nonweighed food diaries were 0.35 ($p = 0.003$), 0.51 ($p < 0.001$), and 0.38 ($p = 0.001$; *see* the online supplement).

Parents were also invited to bring the study child to the hospital for an assessment that included spirometry, skin-prick testing, and measurement of exhaled nitric oxide (F_{ENO}). Bronchodilator response was not included in the original study protocol but was introduced for the last 510 children. F_{ENO} measurements were not included in the original study protocol but were included in the last 262 assessments after a methodologic study demonstrated that F_{ENO} measurements could be obtained in 65% of children aged 5 yr with good reproducibility (23). The Grampian Research Ethics Committee approved the study and written parental consent was obtained.

Spirometry and Bronchodilator Response

Spirometry was measured using a pneumotachograph (Spirotrac IV version 4.22; Vitalograph, Maids Moreton, UK) with onscreen incentive software. In accordance with recent suggestions (24), spirometric values presented were the best from at least two technically acceptable expiratory maneuvers; where the ratio of back-extrapolated volume to FVC was less than 5%, there was a rapid rise to peak expiratory flow and smooth descent of the flow–volume curve, and forced expiratory time exceeded 0.5 s. Bronchodilator response was expressed as percentage change in FEV_1 15 min after inhalation of 400 μg albuterol delivered from a metered dose inhaler via a spacer device.

Skin-Prick Testing

Skin-prick reactivity to the allergens cat, timothy grass, egg, and house dust mite (ALK, Hungerford, UK) was determined. The negative control was 0.9% saline and the positive control was histamine 10 mg/ml. A positive response was defined as a mean weal diameter 3 mm or greater than the negative control. Atopy was defined as at least one positive response.

Measurements of F_{ENO}

An NIOX analyzer (Aerocrine, Solna, Sweden) was used to measure F_{ENO} after spirometry and bronchodilator response. F_{ENO} was measured in accordance with international guidelines (25). Up to nine attempts were permitted (20) to obtain mean values from either two measurements within 5% or three within 10% of each other (25).

Statistical Analysis

The primary outcome variables of interest were the prevalence of wheeze and asthma obtained by questionnaire. The primary exposures of interest were maternal antioxidant and trace element intakes and their plasma concentrations at 12 wk gestation and delivery. Maternal and children's dietary and supplement intakes were summated to give total nutrient intake, energy adjusted, and divided into fifths (26). The quintiles of nutrient intake were derived from all of the women completing the FFQ and not merely those responding at 5 yr. Univariate associations between dependent and independent variables were assessed with Mantel-Haenszel odds ratios, multivariate analysis being performed by logistic regression with adjustment for the following covariates: maternal age, maternal atopy, maternal smoking, maternal vitamin C intake, maternal vitamin E or zinc intake, father's social class, maternal age of leaving full-time education, deprivation index based on area of residence, birth weight, birth head circumference, birth crown–heel length, child's sex, birth order, breast feeding, and use of antibiotics by child in first year of life. Separate analyses replaced maternal smoking with number of smokers in the 5-yr-old child's house; however, inclusion of this parameter did not change the nature or the strength of the associations reported below. Plasma α-tocopherol was adjusted for plasma cholesterol (27). The wheezing data for the children at 5 yr were combined with data obtained previously when the children were 6, 12, and 24 mo (16) to classify the children into longitudinal wheezing phenotypes analogous to those used in other birth cohorts—namely, never wheezed, early transient (wheezed 0–2, not at 5), late-onset (no wheeze 0–2, wheeze at 5), and early persistent wheezers (wheeze 0–2 and at 5 yr) (28, 29). Eczema and diagnosed asthma outcomes were also similarly longitudinally categorized. Multinomial logistic regression with adjustment for the covariates listed above related the combined longitudinal variables to maternal dietary antioxidant and trace element intakes during pregnancy. Similar analyses related children's nutrient intake to respiratory outcomes.

F_{ENO} values were log-normally distributed. The χ^2, Student's *t* test, and analysis of variance were used to compare differences between groups where appropriate. Multivariate models were used to determine the relationship between maternal antioxidant intake during pregnancy and childhood asthma, atopy, spirometry, and F_{ENO}. Analyses were done using SPSS version 13.0 (SPSS, Inc., Chicago, IL).

RESULTS

Subjects

Of the 2,000 pregnant women originally recruited, 1,861 were contacted for the present study; of the remaining 139 mothers, 56 had no follow-up address, 41 had withdrawn from the study, and 42 had either delivered twins or had stillbirths. The FFQ was completed by 1,704 (91.6%) mothers. Plasma antioxidant concentrations were measured in 1,856 (99.7%) mothers at 12 wk gestation, 1,134 (60.9%) mothers at delivery, and in 877 (47.1%) cord blood samples. Symptom questionnaire data were obtained for 1,253 children (67%), with dietary data being available for 1,120 (89%) and 797 (64%) children attended for hospital assessment. All of the 797 children attempted to perform spirometry; 639 (80%) were successful and 478 children were able to provide a prebronchodilator FEV_1 measurement. Of 502 children who attempted post-bronchodilator spirometry, 383 (76%) were successful and 269 children were able to provide a post-bronchodilator measurement. Skin-prick reactivity and F_{ENO} were determined in 700 and 167 children, respectively.

Tables 1 and 2 demonstrate that the mothers who responded to the 5-yr questionnaire were less likely to smoke, were older, of higher socioeconomic status, less likely to have wheezed or to have had asthma, and their plasma ascorbate and α-tocopherol levels were higher than women who failed to respond. The participating 5-yr-old children were slightly larger at birth and more likely to have been delivered by caesarean section and breast fed. The mothers and children who attended for hospital assessment were broadly representative of those responding to the questionnaire, although the mothers were more likely to have wheezed, to be using asthma medication, and to have breast fed the child. The children who were skin-prick tested, or who had successful measurements of F_{ENO}, pre-, and post-bronchodilator FEV_1 were

TABLE 1. CHARACTERISTICS OF MOTHERS AT RECRUITMENT, THOSE RESPONDING TO 5-YEAR QUESTIONNAIRE, AND THOSE WHO BROUGHT CHILD FOR HOSPITAL ASSESSMENT

Maternal Characteristics	All Mothers of Singleton Child (*n = 1,924*)	Respondent Mothers at 5 yr (*n = 1,253*)	p*	Mothers of Children Assessed in Hospital (*n = 797*)	p†
Maternal age at recruitment, mean, yr (95% CI)	28.9 (28.6–29.1)	29.9 (29.6–30.2)	< 0.001	30.0 (29.6–30.3)	0.34
Current smoker at recruitment, n (%)	566 (29.4)	288 (23.0)	< 0.001	176 (22.1)	0.28
Partner's social class nonmanual, n (%)	1,119 (58.2)	767 (61.2)	< 0.001	489 (61.4)	0.89
Age left full-time education, median, yr (IQR)	18.3 (16.0–21.0)	18.5 (16.0–21.0)	< 0.001	18.4 (16.0–21.0)	0.86
Obtained a degree, %	24.1	27.5	< 0.001	27.5	0.99
Ever wheezed, n (%)	708 (36.8)	415 (33.1)	< 0.001	282 (35.4)	0.02
Asthma ever, n (%)	316 (16.4)	189 (15.1)	0.01	131 (16.4)	0.08
Asthma medication, n (%)	203 (10.6)	123 (9.8)	0.07	90 (11.3)	0.02
Ever eczema, n (%)	314 (16.3)	213 (17.0)	0.39	141 (17.7)	0.39
Ever hayfever, n (%)	476 (24.8)	313 (25.0)	0.75	205 (25.7)	0.42
Atopic, n (%)	689 (35.8)	448 (35.8)	0.94	300 (37.6)	0.07
First pregnancy, n (%)	656 (35.8)	437 (36.7)	0.67	279 (36.1)	0.78
FFQ returned, n (%)	1,717 (89.2)	1,208 (96.7)	< 0.001	779 (97.7)	0.01
Dietary supplement use, %	44.7	45.6	0.27	44.6	0.34
Vitamin C intake, mg/d, geometric mean (95% CI)	119 (115.6–122.0)	120 (116.6–123.8)	0.19	119 (114.5–123.0)	0.28
Vitamin E intake, mg/d, geometric mean (95% CI)	8.20 (8.02–8.39)	8.14 (7.93–8.36)	0.31	8.01 (7.77–8.26)	0.11
Zinc intake, mg/d, geometric mean (95% CI)	12.5 (12.2–12.7)	12.4 (12.1–12.7)	0.57	12.3 (12.0–12.6)	0.25
Plasma α-tocopherol/cholesterol, μg/mmol, mean (95% CI)	2.04 (2.01–2.06)	2.06 (2.03–2.09)	0.02	2.07 (2.03–2.11)	0.18
Plasma vitamin C, μmol/L geometric mean (95% CI)	61.2 (59.8–62.7)	64.2 (62.5–65.9)	< 0.001	64.4 (62.4–66.6)	0.71
Plasma zinc C, μmol/L, geometric mean (95% CI)	15.5 (15.3–15.6)	15.5 (15.3–15.7)	0.48	15.4 (15.2–15.6)	0.19

Definition of abbreviations: CI = confidence interval; FFQ = Scottish Collaborative Group Food Frequency Questionnaire; IQR = interquartile range.
* p value: responders at 5 yr versus nonresponders.
† p value: responders at 5 yr, attended hospital versus didn't attend hospital.

representative of the children who attended for hospital assessment; there were, however, minor differences in plasma vitamin C and or zinc concentrations at 12 wk gestation in the mothers of children who successfully had FE_{NO} or post-bronchodilator FEV_1 measurements (*see* Tables E1 and E2 of the online supplement).

Maternal Nutrient Intake and Outcomes in 5-Yr-Old Children

The prevalence of wheezing symptoms, asthma, eczema, hayfever, and atopic sensitisation in the 5-yr-old children is outlined in Table 3. There were no consistent associations between wheezing symptoms, asthma, eczema, hayfever, and atopic sensitization in 5-yr-old children and maternal intakes of vitamin C, β-carotene, magnesium, copper, and iron during pregnancy. Univariate analysis highlighted a number of negative associations between maternal manganese intake and wheeze, asthma, and hayfever outcomes in 5-yr-old children; however, these associations were insignificant after adjustment for confounding factors. Maternal use of nutritional supplements during pregnancy was not associated with any of the outcomes measured in 5-yr-old children.

Vitamin E

There were negative associations between maternal vitamin E intake during pregnancy assessed at 32 wk gestation and wheezing (in previous year, in absence of a cold, consulting doctor for) and asthma outcomes (ever, doctor confirmed, with wheeze in previous year) in children aged 5 yr (Table 4). Maternal vitamin E intake was not associated with eczema or atopic sensitization in 5-yr-old children. Although breast feeding of an infant was not associated with 5-yr outcomes, analysis of the children who had (74.6%), and had not been breast fed suggested that the associations between maternal vitamin E intake and wheeze/asthma outcomes in 5-yr-old children were of greater magnitude

TABLE 2. CHARACTERISTICS OF THE COHORT OF CHILDREN, THOSE WITH 5-YEAR QUESTIONNAIRE DATA, AND THOSE ATTENDING FOR HOSPITAL ASSESSMENT

Infant Variable	All Children (*n = 1,924*)	Responders at 5 yr (*n = 1,253*)	p*	Children Assessed in Hospital (*n = 797*)	p†
Male, n (%)	968 (50.3)	630 (50.3)	1.00	400 (50.2)	0.93
Birth weight, g, mean (95% CI)	3,436 (3,411–3,462)	3,458 (3,426–3,489)	0.13	3,438 (3,398–3,478)	0.13
Crown–heel length, cm, mean (95% CI)	49.8 (49.7–50.0)	49.9 (49.8–50.1)	0.02	49.8 (49.7–50.0)	0.094
Head circumference, cm, mean (95% CI)	34.8 (34.7–34.9)	34.9 (34.8–35.0)	0.05	34.8 (34.7–34.9)	0.06
Caesarian section, %	21.7	23.4	0.01	23.8	0.70
Cord, α-tocopherol/cholesterol, μg/mmol, mean (95% CI)	0.98 (0.91–1.05)	1.01 (0.92–1.11)	0.20	1.01 (0.90-1.11)	0.90
	(*n = 871*)	(*n = 596*)			
Cord vitamin C, μmol/L, geometric mean (95% CI)	78.7 (76.1–81.5)	79.7 (76.6–82.9)	0.31	80.9 (77.0–85.1)	0.30
	(*n = 883*)	(*n = 607*)			
Ever breast fed, %	70.9	74.3	< 0.001	77.6	0.001
Breast fed > 4 mo, %	57.4	62.3	< 0.001	66.5	0.001

Definition of abbreviation: CI = confidence interval.
*p value: responders at 5 vs non-responders.
†p value: responders at 5, attended hospital vs didn't attend hospital.

TABLE 3. PREVALENCE OF WHEEZE, SHORTNESS OF BREATH, ASTHMA, ECZEMA, HAYFEVER, AND ATOPIC SENSITIZATION IN 5-YEAR-OLD CHILDREN

	Responders at 5 yr (*n = 1,253*)		Responders at 5 yr (*n = 1,253*)
Ever wheezed, n (%)	254 (20.3)	Ever short of breath, n (%)	117 (9.3)
Wheezed in last 12 mo, n (%)	162 (12.9)	Short of breath in last 12 mo, n (%)	79 (6.3)
Wheeze in the absence of a cold in last 12 mo, n (%)	84 (6.7)	Short of breath in absence of a cold in last 12 mo, n (%)	47 (3.8)
Wheezing most of the time in last 12 mo, n (%)	18 (1.4)	Short of breath most of the time in last 12 mo, n (%)	5 (0.4)
Seen doctor because of wheeze in last 12 mo, n (%)	119 (9.5)	Seen doctor because of shortness of breath in last 12 mo, n (%)	56 (4.5)
Ever had asthma, n (%)	157 (12.6)		
Doctor-confirmed asthma, n (%)	146 (11.7)		
Current treatment for asthma, n (%)	145 (11.6)	Positive skin-prick test to	
Asthma and wheeze in previous 12 mo, n (%)	107 (8.6)	Cat, n (%)	54/700 (7.7)
Doctor-confirmed eczema, n (%)	359 (29.1)	House dust mite, n (%)	71/700 (10.1)
Current treatment for eczema, n (%)	191 (15.4)	Hen's egg, n (%)	9/700 (1.3)
Doctor-confirmed hayfever, n (%)	54 (4.3)	Timothy grass, n (%)	72/700 (10.3)
Current treatment for hayfever, n (%)	44 (3.5)	Atopic, n (%)	149/700 (21.3)

in breast-fed children; there was no interaction between maternal vitamin E intake and breast feeding (Table E3). Maternal plasma α-tocopherol (corrected for cholesterol) at 12 wk gestation was negatively associated with atopic sensitization in 5-yr-old children (adjusted odds ratio [OR], 0.60 per μg α-tocopherol/mmol cholesterol; 95% confidence interval [CI], 0.40–0.91; $p = 0.02$). There were no other consistent associations between symptomatic outcomes in the 5-yr-old children and plasma levels of ascorbate, β-carotene, α-tocopherol, and zinc, at 12 wk gestation and at delivery (maternal and cord blood).

Maternal vitamin E intake during pregnancy was negatively associated with the persistent wheezing phenotype (wheezing 0–2 yr and 5 yr; adjusted OR per intake quintile, 0.77; 95% CI, 0.63–0.93; $p < 0.01$), with children born to mothers with the lowest quintile of vitamin E intake being 3.47 times (95% CI, 1.38–8.72; $p < 0.01$) more likely to be of the persistent wheezing phenotype than children born to mothers with the highest quintile of vitamin E intake. Maternal vitamin E intake during pregnancy was negatively associated with early persistent asthma (onset < 2 and present at 5 yr; adjusted OR per intake quintile, 0.67; 95% CI, 0.51–0.90, $p < 0.01$). Children born to mothers from the lowest quintile of vitamin E intake were 5.14 times (95% CI, 1.49–17.7; $p < 0.01$) more likely to be of the early persistent asthma phenotype than children born to mothers from the highest quintile of vitamin E intake.

The geometric mean F_{ENO} for 167 5-yr-old children was 6.5 parts per billion (ppb; 95% CI, 5.9–7.1). Exhaled NO was negatively associated with maternal vitamin E intake (p [trend] = 0.02; Figure 1). There was no significant interaction by maternal atopy for the association between maternal vitamin E intake and F_{ENO}. Maternal plasma α-tocopherol at delivery (but not at 12 wk gestation) was negatively correlated with F_{ENO} but only among children with atopic mothers ($p = 0.02$; Figure 2); a multivariate model confirmed an interaction term for increased F_{ENO} between maternal atopy and reduced maternal α-tocopherol at delivery that was independent of doctor-diagnosed asthma ($p = 0.03$).

In bivariate correlation, maternal plasma α-tocopherol at 12 wk gestation was positively correlated with $FEV_{0.5}$ (pre- and post-bronchodilator), $FEV_{0.75}$ (pre- and post-bronchodilator), FEV_1 (post-bronchodilator), and FVC (pre- and post-bronchodilator); there were also similar relationships for FEV_1 (prebronchodilator) and PEF (prebronchodilator) that approached borderline statistical significance (p values of 0.07 and 0.06, respectively). Multivariate linear regression with adjustment for potential confounders (including height, weight, sex, maternal atopy, breast feeding, maternal smoking, and plasma cholesterol) demonstrated that when compared with children born to mothers with the highest tertile of plasma α-tocopherol at 12 wk gestation, the post-bronchodilator FEV_1 of children born to mothers with the middle tertile of plasma α-tocopherol was reduced by 65 ml (95% CI, 19–111; $p < 0.01$), and the post-bronchodilator FEV_1 of children born to mothers with the lowest tertile of plasma α-tocopherol was reduced by 77 ml (95% CI, 26–128; $p < 0.01$). The mean (SD) plasma α-tocopherol values at 12 wk gestation for the tertiles were as follows: lowest, 7.03 μg/ml (1.57); middle, 10.1 μg/ml (0.61); and highest, 13.2 μg/ml (1.86). Inclusion of log (height) or $(height)^2$ in the models did not alter the association between maternal plasma α-tocopherol at 12 wk gestation and post-bronchodilator FEV_1 at 5 yr. Maternal vitamin E intake during pregnancy and maternal plasma α-tocopherol at delivery were not related to ventilatory function. The median bronchodilator response was 4.3% (interquartile range, 0.0–8.4; $n = 238$). No relationships were apparent between maternal nutrient status and bronchodilator response.

Maternal plasma α-tocopherol concentrations at 12 wk gestation and at delivery were weakly correlated, with Spearman correlation coefficients of 0.21, $p < 0.001$ with, and 0.34, $p < 0.001$ without, adjustment for plasma cholesterol. There were no associations between changes in maternal plasma α-tocopherol concentration during pregnancy and any of the measured outcomes in the 5-yr-old children.

Zinc

Maternal zinc intake during pregnancy was negatively associated with shortness of breath (in previous year, in absence of a cold), asthma (ever, with wheeze in previous year) and eczema (ever, doctor confirmed, current treatment for) in 5-yr-old children (Table 5). Zinc intake was not associated with atopic sensitization in 5-yr-old children. Analysis of the children who had and had not been breast fed suggested that the associations between maternal zinc intake and dyspnea/asthma outcomes in 5-yr-old children were of greater magnitude in breast-fed children; there was no interaction between maternal zinc intake and breast feeding (Table E4). There were no significant associations between any of the measured 5-yr outcomes in children and maternal plasma zinc concentrations (12 wk gestation and delivery) and cord plasma zinc concentrations. Maternal plasma zinc concentrations at 12 wk gestation and delivery were very weakly negatively correlated with a Spearman correlation coefficient of –0.13, $p < 0.001$. There were no associations between changes in maternal plasma zinc concentration during pregnancy and any of the measured outcomes in the 5-yr-old children.

TABLE 4. ASSOCIATIONS BETWEEN TOTAL MATERNAL VITAMIN E INTAKE DURING PREGNANCY AND WHEEZING AND ASTHMA OUTCOMES IN 5-YEAR-OLD CHILDREN

Quintiles of Vitamin E Intake*	Unadjusted OR (*95% CI*)	p (Trend)	Adjusted OR† (*95% CI*)	p (Trend)
Ever wheeze				
1st (lowest)	1		1	
2nd	0.75 (0.49–1.17)	0.06	0.84 (0.52–1.37)	0.07
3rd	0.80 (0.52–1.23)		0.92 (0.57–1.50)	
4th	0.48 (0.30–0.76)		0.50 (0.29–0.85)	
5th (highest)	0.49 (0.50–1.18)		0.75 (0.44–1.28)	
Wheeze in last 12 mo				
1st (lowest)	1		1	
2nd	0.69 (0.42–1.13)	0.006	0.76 (0.43–1.35)	0.01
3rd	0.65 (0.40–1.07)		0.75 (0.42–1.34)	
4th	0.43 (0.24–0.74)		0.51 (0.27–0.95)	
5th (highest)	0.56 (0.33–0.94)		0.46 (0.24–0.90)	
Wheeze without cold in last 12 mo				
1st (lowest)	1		1	
2nd	0.58 (0.29–1.17)	0.06	0.50 (0.22–1.15)	0.01
3rd	0.64 (0.33–1.25)		0.56 (0.25–1.28)	
4th	0.54 (0.26–1.09)		0.53 (0.23–1.23)	
5th (highest)	0.49 (0.24–1.01)		0.22 (0.08–0.62)	
Seen doctor with wheeze in last 12 mo				
1st (lowest)	1		1	
2nd	0.62 (0.35–1.11)	0.08	0.80 (0.42–1.54)	0.02
3rd	0.62 (0.35–1.10)		0.75 (0.39–1.47)	
4th	0.47 (0.25–0.87)		0.58 (0.29–1.18)	
5th (highest)	0.49 (0.27–0.91)		0.38 (0.17–0.87)	
Ever asthma				
1st (lowest)	1		1	
2nd	0.66 (0.39–1.10)	0.02	0.64 (0.35–1.16)	0.04
3rd	0.57 (0.341–0.976)		0.60 (0.33–1.12)	
4th	0.53 (0.31–0.90)		0.59 (0.32–1.10)	
5th (highest)	0.57 (0.34–0.97)		0.47 (0.24–0.92)	
Doctor-confirmed asthma				
1st (lowest)	1		1	
2nd	0.60 (0.35–1.02)	0.02	0.54 (0.29–0.99)	0.02
3rd	0.554 (0.32–0.94)		0.53 (0.28–0.99)	
4th	0.44 (0.25–0.79)		0.48 (0.25–0.93)	
5th (highest)	0.59 (0.35–1.00)		0.45 (0.23–0.89)	
Asthma and wheeze in last 12 mo				
1st (lowest)	1		1	
2nd	0.60 (0.33–1.10)	0.02	0.56 (0.27–1.14)	0.02
3rd	0.61 (0.332–1.103)		0.58 (0.28–1.198)	
4th	0.53 (0.280–1.986)		0.61 (0.30–1.26)	
5th (highest)	0.46 (0.24–1.88)		0.28 (0.11–0.69)	

Definition of abbreviations: CI = confidence interval; OR = odds ratio.

* Quintiles of vitamin E intake (mg/d) adjusted for energy intake, 2.61–6.21, 6.21–7.07, 7.07–7.89, 7.90–9.15, 9.17–30.8.

† Adjusted for maternal age, maternal atopy, maternal smoking, maternal vitamin C intake, maternal zinc intake, father's social class, maternal age of leaving full-time education, deprivation index, birth weight, birth head circumference, birth crown–heel length, child's sex, birth order, breast feeding, and use of antibiotics by child in first year of life.

Maternal zinc intake during pregnancy was negatively associated with late-onset asthma (onset > 2; adjusted OR per intake quintile, 0.82; 95% CI, 0.68–0.99; p = 0.04). Children born to mothers from the lowest quintile of zinc intake were 1.91 times (95% CI, 0.86–4.22) more likely to be of the late-onset asthma phenotype than children born to mothers from the highest quintile of zinc intake. Maternal zinc intake during pregnancy was negatively associated with the late eczema phenotype (no eczema, 0–2 yr; eczema at 5 yr; adjusted OR per intake quintile, 0.84; 95% CI, 0.70–1.00; p = 0.05). No index of maternal zinc status during pregnancy (intake or plasma) was related to F_{ENO} or ventilatory function in the 5-yr-old children.

Five-Year-Old Children's Nutrient Intake and Outcomes

Maternal intake of vitamin E, vitamin C, and zinc during pregnancy were very weakly positively associated with children's intake at 5 yr with rank correlation coefficients between energy-adjusted intakes of maternal and children's intakes of 0.08 (p = 0.02) for vitamin E, 0.14 (p < 0.001) for vitamin C, and 0.09 (p < 0.01) for zinc. There were no consistent associations between wheezing, dyspnea, asthma, eczema, hayfever, and atopic sensitisation in 5-yr-old children and their intakes of vitamin E, vitamin C, and zinc (Tables E5 and E6).

DISCUSSION

We have previously reported negative associations between maternal vitamin E intake during pregnancy and cord blood mononuclear cell responses at birth (30) and wheeze and eczema in these children at age 2 (16). In the present follow-up of the cohort, we report that low maternal vitamin E intake during pregnancy is associated with increased likelihood of wheezing and asthma in 5-yr-old children and with the persistent wheezing and persistent asthma phenotypes during the first 5 yr of life. In a representative subgroup of children, we report that low maternal vitamin E intake during pregnancy and low maternal plasma α-tocopherol at delivery are associated with increased

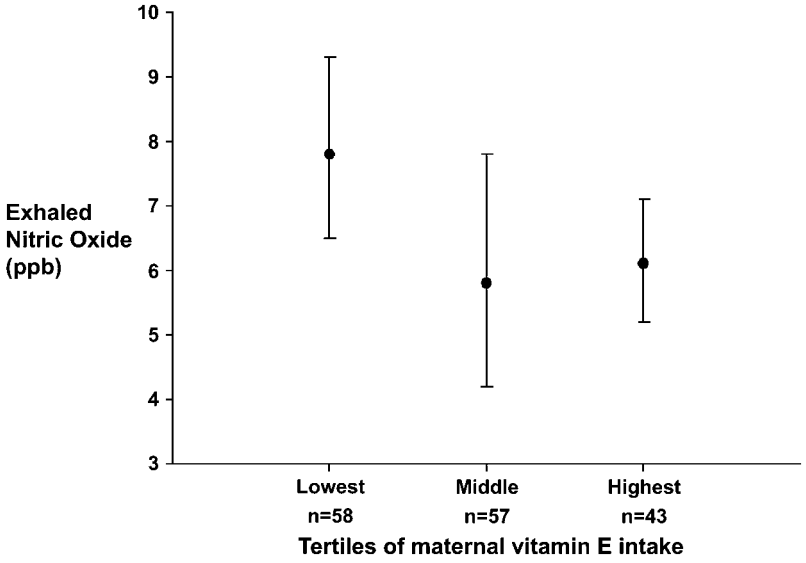

Figure 1. Mean (95% confidence interval) values for exhaled nitric oxide in children categorized by tertiles of maternal vitamin E intake at 32 wk gestation. p (trend) = 0.02.

FE_{NO} in 5-yr-old children. In the subgroup of children, high maternal α-tocopherol at 12 wk gestation is associated with increased post-bronchodilator FEV_1. Although we demonstrated an association between high maternal plasma α-tocopherol at 12 wk gestation and a reduced likelihood of atopic sensitization in the children at 5 yr, we cannot exclude the possibility that this was a chance finding because maternal plasma α-tocopherol at this early stage of pregnancy was not associated with any symptomatic outcomes at 5 yr. This association is, however, consistent with reports relating vitamin E to atopic sensitization in adults (31, 32).

In observational studies, beneficial associations have been reported between vitamin E/α-tocopherol and ventilatory function, asthma, adult-onset wheeze, serum IgE, and atopic sensitization (3–5, 31, 32). However, vitamin E supplementation in adults with established asthma is not associated with any clinical benefit (14). A small number of observational studies have reported negative associations between dietary or plasma/hair zinc levels and seasonal allergic symptoms, wheeze, and asthma (8–11). We are unaware of any zinc supplementation studies in subjects with asthma. A possible explanation for the inconsistencies between epidemiologic and dietary intervention studies is that dietary antioxidants may exert their greatest effects on the pathogenesis of asthma during a critical period of during early life. The results of this study are consistent with the notion that early life nutrient intake, both *in utero* and in the early postpartum period, modifies the risk of developing childhood asthma.

Maternal nutrient intake during pregnancy could modulate the development of asthma by influencing fetal airway development. In rat models of fetal hypoplastic lung growth, maternal vitamin E supplementation accelerates growth in hypoplastic lung, increasing lung complexity, surface area, and bud count (40). Zinc deficiency in pregnant rats is associated with impaired fetal lung growth (41). A disintegrin and metalloprotease 33 (ADAM33) is a zinc-dependent metalloproteinase (42) identified as a putative asthma susceptibility gene (43). ADAM33 expression is induced in embryonic lungs, increases with gestation, and remains present into adulthood (44, 45), and has been

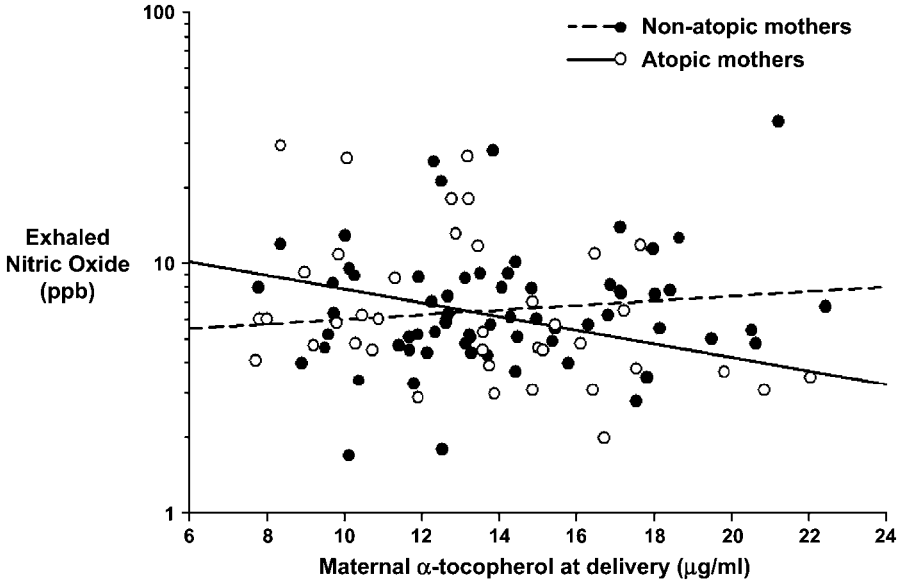

Figure 2. Scatter plots comparing exhaled nitric oxide in 5-year-old children with maternal plasma α-tocopherol concentration at delivery for children with atopic and nonatopic mothers. Spearman's correlation coefficients: atopic mothers, −0.38; p = 0.02; nonatopic mothers, 0.14; p = 0.24.

TABLE 5. ASSOCIATIONS BETWEEN TOTAL MATERNAL ZINC INTAKE DURING PREGNANCY AND SHORTNESS OF BREATH, ASTHMA, AND ECZEMA OUTCOMES IN 5-YEAR-OLD CHILDREN

Quintiles of Zinc Intake*	Unadjusted OR (*95% CI*)	p (trend)	Adjusted OR† (*95% CI*)	p (trend)
Short of breath in last 12 mo				
1st (lowest)	1		1	
2nd	0.88 (0.44–1.73)	0.06	0.92 (0.42–2.01)	0.05
3rd	0.58 (0.27–1.23)		0.57 (0.24–1.37)	
4th	0.76 (0.38–1.53)		0.73 (0.31–1.70)	
5th (highest)	0.46 (0.21–1.02)		0.39 (0.15–1.04)	
Short of breath in absence of a cold in last 12 mo				
1st (lowest)	1		1	
2nd	0.88 (0.39–2.00)	0.01	1.05 (0.39–2.8)	0.02
3rd	0.43 (0.16–1.18)		0.47 (0.14–1.55)	
4th	0.57 (0.23–1.41)		0.60 (0.18–1.96)	
5th (highest)	0.28 (0.09–0.87)		0.20 (0.04–0.98)	
Ever asthma				
1st (lowest)	1		1	
2nd	0.60 (0.35–1.02)	0.05	0.55 (0.30–1.01)	0.04
3rd	071 (0.42–1.19)		0.59 (0.32–1.07)	
4th	049 (0.28–1.86)		0.44 (0.23–0.85)	
5th (highest)	0.61 (0.36–1.04)		0.51 (0.27–0.97)	
Current treatment for asthma				
1st (lowest)	1		1	
2nd	0.75 (0.43–1.29)	0.09	0.76 (0.41–1.39)	0.08
3rd	0.70 (0.40–1.22)		0.60 (0.32–1.14)	
4th	0.62 (0.35–1.08)		0.54 (0.28–1.05)	
5th (highest)	0.65 (0.37–1.13)		0.61 (0.32–1.17)	
Asthma and wheeze in last 12 mo				
1st (lowest)	1		1	
2nd	0.66 (0.36–1.20)	0.01	0.63 (0.32–1.25)	0.003
3rd	0.63 (0.34–1.16)		0.54 (0.26–1.10)	
4th	0.51 (0.27–0.97)		0.47 (0.22–1.03)	
5th (highest)	0.45 (0.23–0.87)		0.28 (0.12–0.67)	
Ever eczema				
1st (lowest)	1		1	
2nd	0.95 (0.64–1.39)	0.079	0.88 (0.57–1.35)	0.03
3rd	0.85 (0.58–0.25)		0.74 (0.47–1.15)	
4th	0.81 (0.55–1.19)		0.74 (0.47–1.15)	
5th (highest)	0.73 (0.50–1.08)		0.63 (0.40–0.95)	
Doctor-confirmed eczema				
1st (lowest)	1		1	
2nd	0.91 (0.61–1.34)	0.06	0.85 (0.55–1.33)	0.05
3rd	0.95 (0.65–1.407)		0.83 (0.54–1.30)	
4th	0.71 (0.47–1.06)		0.67 (0.42–1.05)	
5th (highest)	0.74 (0.50–1.10)		0.67 (0.43–1.07)	
Current treatment for eczema				
1st (lowest)	1		1	
2nd	0.60 (0.37–0.98)	0.03	0.45 (0.25–0.79)	0.04
3rd	0.75 (0.47–1.20)		0.61 (0.36–1.05)	
4th	0.58 (0.36–0.95)		0.44 (0.25–0.78)	
5th (highest)	0.54 (0.33–0.89)		0.51 (0.29–0.90)	

For definition of abbreviations, *see* Table 4.

* Quintiles of zinc intake (mg/d) adjusted for energy intake, 4.44–10.19, 10.19–11.43, 11.43–12.62, 12.63–14.22, 14.25–30.30.

† Adjusted for maternal age, maternal atopy, maternal smoking, maternal vitamin C intake, maternal vitamin E intake, father's social class, maternal age of leaving full-time education, deprivation index, birth weight, birth head circumference, birth crown–heel length, child's sex, birth order, breast feeding, use of antibiotics by child in first year of life.

implicated in lung growth and development (45). It is possible to speculate that the associations we have demonstrated between maternal zinc intake and childhood asthma may be mediated through ADAM33. If low maternal vitamin E and zinc intakes during pregnancy impair fetal airway development, extrapolation of studies of pulmonary function in early life suggest that these children are more likely to wheeze, develop asthma, and have reduced ventilatory function later in life, particularly if they become atopic (46, 47). In the present study, maternal vitamin E intake was positively associated with a 6–7% change in post-bronchodilator FEV_1, and although the magnitude of this association was small, it is likely to be important to whole populations. Longitudinal tracking of lung function has been reported from infancy into childhood (46) and then adulthood (47); perhaps the associations between vitamin E and ventilatory function reported in studies of older children (3) and adults (48) are a life-long consequence of reduced maternal intake during pregnancy.

Maternal vitamin E and zinc intake during pregnancy and lactation could potentially influence fetal/neonatal Th-cell differentiation. In animal models and in humans, vitamin E and zinc have been reported to promote Th1 differentiation by increasing Th1-cytokine secretion or inhibiting Th2-cytokine secretion (49–52). Human Th cells supplemented with physiologic quantities of vitamin E demonstrate reduced interleukin (IL)-4 secretion in a dose-dependent manner (51). Vitamin E appears to act by down-regulating IL-4 mRNA expression in human Th-cells by inhibiting binding of the transcription factors nuclear factor-κB

and activator protein (AP)-1 to the IL-4 promoter region (51). Immunologic considerations of Th-cell differentiation suggest that nutrients should exert their most potent influences on Th-cell polarization during the earliest exposures of the immune system to allergens—that is, during fetal and early life. In a subgroup of 223 children from the cohort reported here, we have demonstrated associations between maternal vitamin E intake during pregnancy and neonatal Th-cell responses (30). Our findings suggest that vitamin E has a dual effect on lung function and airway inflammation and that the effects of vitamin E could change at differing periods of prenatal and early life; lung function was associated with early vitamin E exposure independent of atopy whereas allergic airway inflammation was associated with vitamin E exposure in later pregnancy. The immune system is at an early stage of development in the first trimester but term infants are born with a sophisticated immune system (53, 54); this could account for the lack of association between maternal plasma α-tocopherol at 12 wk gestation and F_{ENO}. In contrast, the airways are fully developed by 16 wk after conception and thus vitamin E exposure in early pregnancy may be more likely to influence airway function than exposure later in pregnancy.

The associations between maternal nutrient intake during pregnancy and childhood outcomes highlight the possible importance of maternal nutrient intake during pregnancy, whereas the suggestion that these associations are greater in magnitude in breast-fed children raises the possibility that maternal nutrient intake during the period of breast feeding may also influence the development of childhood asthma. The present study suggests that children's nutrient intake at the age of 5 yr does not modify the associations between maternal nutrient intake and respiratory outcomes in children. Maternal and childhood intakes of vitamin C, vitamin E, and zinc were only very weakly correlated and there were no associations between respiratory outcomes in 5-yr-old children and their intake of vitamin E and zinc.

The original study population of 2,000 pregnant women was very similar demographically to the local obstetric population (16), but there has been some loss to follow-up with time. Although we found no significant difference in maternal vitamin E intake between mothers who did or did not respond, plasma cholesterol corrected α-tocopherol levels were higher in responders. The profile of the women failing to respond at 5 yr is consistent with other studies that have found lower blood antioxidant levels, and increased respiratory symptoms, in subjects from lower socioeconomic groups (33–35). Although there was evidence of typical response biases among those participating in this study, it is unlikely that these could account for the observed associations with maternal vitamin E intake because the nature of the biases would be to weaken the observed negative associations rather than to augment them. FFQ-derived estimates of dietary nutrient intake are reliant on subject recall of the actual foods consumed and of their quantities. It is unlikely that dietary misreporting has contributed to spurious significant associations because the misclassification of portion sizes and intake with respect to disease outcome is usually random and tends to attenuate associations (36). For these reasons, the moderate associations reported here are likely to be underestimates of the true association. Although we adjusted for variables linked to the hygiene hypothesis (37) and socioeconomic status, we cannot eliminate the possibility that the observed associations between vitamin E and zinc intakes and wheeze and asthma outcomes are a consequence of residual confounding by factors associated with a higher socioeconomic status and a healthy lifestyle. It is possible that the lack of association between childhood zinc intake and outcomes reflected the fact that the FFQ used in the children (validation correlation coefficient, 0.38) was less good in quantifying zinc intake than the FFQ used for the mothers (validation correlation coefficient, 0.57). However, this seems unlikely for vitamin E for which the correlation coefficients obtained when validating the maternal and children's FFQ were very similar (0.52 and 0.51, respectively).

In an attempt to reduce the chance of type 1 errors, we restricted the nutrients studied to those that could be reliably estimated by the FFQ, those that had shown associations in these children at 2 yr, those that are antioxidants, or to those that have been associated with asthma outcomes in epidemiologic studies. Major sources of vitamin E in the U.K. diet include vegetable oils (sunflower, rapeseed, corn), margarine, wheat germ, nuts, and sunflower seeds, while those for zinc include liver, wheat germ, lean red meat, seeds, and nuts. Although maternal intakes of vitamin E and zinc were weak/moderately associated (correlation coefficient between quintiles of vitamin E and zinc intake, 0.42; $p = 0.02$), the reported associations remained significant after mutual adjustment. In addition, maternal vitamin E and zinc intakes were associated with different patterns of 5-yr outcomes in the children. We were not able to investigate possible associations between maternal n-3 and n-6 fatty acid intakes and symptoms in the children, as this information is not currently available for all foods in U.K. food composition tables. The associations reported here with vitamin E are consistent within this study and with other published studies (3–5, 16, 30). Although the associations reported here with maternal zinc intake are not as consistent (with symptoms only) as for vitamin E, given the number of associations and biological plausibility it is difficult to ignore them.

In contrast to the 2-yr follow-up of this cohort (16), measurements of ventilatory function and F_{ENO} were obtained in a representative subgroup of children. Measurements of F_{ENO} were incorporated into the study after a feasibility study in this young age group (23). F_{ENO} measurements in this study were directly comparable with values obtained in the feasibility study that followed international guidelines (23), and the expected associations between F_{ENO}, atopic sensitization, and doctor-diagnosed asthma were present in the current study (*see* the online supplement). Other groups have reported comparable F_{ENO} values (38, 39) in similar age groups.

The cohort study reported here has highlighted associations between maternal vitamin E intake during pregnancy and neonatal immune responses (30), wheezing at 2 yr, (16), and now wheezing, asthma, ventilatory function, and F_{ENO} at 5 yr. Further follow-up of this cohort is required to determine whether associations with maternal diet persist into later childhood. The results of the present study suggest that dietary modification or supplementation during pregnancy to reduce the likelihood of childhood asthma warrants further investigation.

***Conflict of Interest Statement*:** None of the authors has a financial relationship with a commercial entity that has an interest in the subject of the manuscript.

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