

Antioxidant Intake in Pregnancy in Relation to Wheeze and Eczema in the First Two Years of Life

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Two thousand women were recruited for a prospective investigation of the influence of maternal antioxidant intake in pregnancy on the development of asthma and eczema in children. A food frequency questionnaire was used to characterize diet during pregnancy and blood antioxidant levels were measured. Postal questionnaires were used to follow up the 1,924 singleton children born to the cohort at 6, 12, and 24 months of age. There were no associations between maternal antioxidant intake and wheezing symptoms and eczema in the children's first year. In the children's second year, maternal vitamin E intake during pregnancy was negatively associated with wheeze in the absence of a "cold" (p for trend 0.010) and, in children whose mothers were atopic, there was a negative association between maternal vitamin E intake and childhood eczema (p for trend 0.024). Maternal vitamin C intake during pregnancy was positively associated with "ever wheeze" and eczema during the children's second year. This study suggests that maternal dietary antioxidant intakes during pregnancy may modify the risks of developing wheeze and eczema during early childhood. Further follow up of the cohort will determine whether maternal diet during pregnancy is associated with asthma and atopic disease in later childhood.

Keywords: asthma; atopy; children; diet; pregnancy

In the final decades of the twentieth century, prosperous countries witnessed increases in the prevalence of the atopic diseases asthma, eczema, and hay fever (1–5), especially among children. For example, in Aberdeen the percentage of parents reporting that their child had ever had asthma increased from 4.1% in 1964 to 24.3% in 1999 (6). Such rapid increases are most likely to be a consequence of changing environmental influences, and it has been suggested that these trends in asthma and atopic disease are a consequence of altered patterns of childhood infection (7). An alternative proposal is that changes in the population's diet have played a role (8). In the United Kingdom, the increases in asthma and atopic disease were preceded and paralleled by major changes in diet, with a decline in vegetable consumption, particularly potatoes and fresh green vegetables, and a shift from animal to vegetable fats (9). These dietary trends are likely to be associated with changes in antioxidant status that could increase population susceptibility to airway oxidative stresses with a consequent increase in asthma prevalence (8). In addition

to ameliorating oxidative stress, many antioxidants have effects on immune function, with *in vitro* data being consistent with the suggestion that relative dietary antioxidant deficiency may influence T-helper (Th) cell differentiation toward the Th2 (atopic) phenotype (10–12). Epidemiologic studies have demonstrated beneficial effects of the naturally occurring dietary antioxidants vitamin C, vitamin E, β -carotene, selenium, and foods rich in antioxidants on ventilatory function (13–20), respiratory symptoms (17, 21–29), and atopy (30, 31) in children and adults.

In recent years there has been increasing interest in the possibility that factors operating before birth may influence the development of atopic disease in children and possibly even adults (32). Gestational age and neonatal anthropometric measurements are associated with atopic disease in children and adults (33, 34). Differences have also been reported between the *in vitro* responses of cord blood T-helper cells from neonates who subsequently develop atopic disease and those of healthy control subjects (35). The nature of influential environmental antenatal factors is unclear, but several lines of evidence suggest that maternal dietary antioxidant intake during pregnancy may be a relevant antenatal influence. Dietary vitamin E intake during pregnancy has been shown to be negatively associated with *in vitro* cord blood Th-cell proliferative responses after stimulation with allergens. The increase in Th-cell secretion of the Th1 cytokine interferon- γ reported with dietary vitamin E supplementation is more pronounced in Th-cells that have not been previously stimulated (CD45RA^{high}); these Th-cells predominate in fetal and neonatal blood (11). These studies suggest that maternal vitamin E intake during pregnancy has the potential to influence postnatal susceptibility to asthma and atopic disease by modulating fetal and neonatal Th-cell responses during initial encounters with allergen.

The aim of the present study was to test the hypothesis that maternal antioxidant intake during pregnancy influences childhood susceptibility to asthma and atopic disease (8). It is likely that the influences of maternal diet during pregnancy on susceptibility will manifest as the children get older because the onset of disease requires further relevant postnatal environmental exposures (e.g., child's diet, allergen exposure). We report here the results of a birth cohort of children followed up to 2 years of age. Some of the preliminary findings of this study have been previously reported in the form of abstracts (37, 38).

METHODS

Between October 1997 and April 1999, 2,000 healthy pregnant women were recruited at a median gestational age of 12 weeks (IQR 11–13 weeks) while attending their first antenatal clinic appointment at Aberdeen Maternity Hospital (AMH). Two research nurses conducted recruitment and administered a questionnaire based on the European Community Respiratory Health Survey (39) to collect data on parity, gestational age, occupation, education, smoking habit, and family history of atopic disease. Atopic status was ascertained by standard skin prick testing using ALK lancets and solutions (ALK, UK), testing for house dust mite (*Dermaphagoides pteronyssinus*), timothy grass pollen, cat fur, and saline. A positive skin prick test was defined as a

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mean wheal diameter of at least 3 mm greater than the negative control (39). A nonfasting venous blood sample was obtained. The AMH provides obstetric care for the whole population of the Grampian region (520,000) with a total of 7,490 pregnant women registered at the AMH for antenatal care over the time period. There was no formal selection process; instead all women to whom the research nurses had access were approached (2,690 women). The Aberdeen Maternity and Neonatal Databank enabled a comparison of the 2,000 recruited pregnant women with the 5,490 pregnant women who attended the antenatal clinic during the duration of recruitment, but were not approached (4,800) or refused (690) to participate in the study. The Aberdeen Maternity and Neonatal Databank's classification of social economic status is modified because of local circumstances and therefore differs from the standard classification used for our study. The prevalence of smoking in the databank is derived from clinical data and is substantially lower than the prevalence rate obtained by our research nurses, possibly because the women were more honest with the research nurses. Although, using this databank, the demographic characteristics of the study population differ somewhat from our database, the databank enabled valid comparisons of the 2,000 study participants and the 5,490 women not approached or who refused to participate. Using this databank, the study participants were slightly older (mean age 29.4 years, 95% confidence interval [95% CI] 29.1–29.6 years versus 28.6 years, 95% CI 28.4–28.7 years, $p < 0.001$), more likely to be primiparous (50.6% versus 47.0%, $p = 0.009$), less likely to be current smokers (19.1% versus 23.8%, $p < 0.001$) and, where ascertainable, more likely to be from nonmanual social classes defined from partner's occupation (46.3% versus 43.5%, $p = 0.044$).

Maternal diet during pregnancy was quantified by a semiquantitative food frequency questionnaire (FFQ) administered at 34 weeks gestation. Version 5.4 of the Scottish Collaborative Group FFQ was mailed to participating mothers enquiring about dietary intakes of nutrients. The questionnaire is a 145-item, semiquantitative FFQ that also enquired about the use of vitamin and mineral supplements during the previous 3 months. The timing of the FFQ was chosen to avoid the dietary disruption of early pregnancy and to provide an indication of habitual dietary intake in middle and late pregnancy. The effectiveness of this FFQ to elicit accurate data on the dietary intake of foods containing antioxidant vitamins and minerals has been assessed by comparison with 4-day weighed intakes in 40 women of childbearing age. Spearman correlation coefficients for vitamin E, vitamin C, betacarotene, and selenium were 0.52, 0.59, 0.44, and 0.33, respectively (40), in the same range of values as those found in a large American study (41).

At delivery, maternal and cord blood samples were obtained for the measurement of serum antioxidant levels. Plasma for vitamin C analysis was denatured with 10% metaphosphoric acid and stored at -70°C . Plasma concentrations of vitamin E (α -tocopherol) and β -carotene were determined by normal phase high-pressure liquid chromatography (42). Plasma ascorbate concentrations were measured using enzymatic colorimetric assays (43). In total, 1,089 maternal and 814 cord blood samples were obtained.

The cohort of children was followed up by postal questionnaire at 6, 12, and 24 months after birth. Up to two reminders were sent to nonresponders. The questionnaires enquired about symptoms of wheeze (in the presence or absence of a "cold"), breathlessness, rhinitis, and doctor-diagnosed asthma, eczema, or hayfever. The questions used were based on the ISAAC questionnaire (44). The questions relating to eczema were those recommended in the United Kingdom's Diagnostic Criteria for Atopic Dermatitis (45) as detailed below. The questionnaires also enquired about the number of other children in the house, household pets, and infant antibiotic use. The questionnaires used for the 6- and 12-month follow-up related to the previous 6 months, while the 24-month questionnaire focused on the previous 12 months.

Approval for the study was obtained from the Grampian Research Ethics Committee and all mothers gave written consent to take part.

The primary outcome variables of interest were wheeze and eczema. Wheeze within the specified time period came from the question "has your child had wheezing in the chest (but not from the throat or nose) in the last 6 months" (for questionnaires sent at 6 or 12 months), or "in the last 12 months" (for questionnaires sent at 24 months). The other main outcome was eczema, determined by parental report of doctor diagnosed eczema and/or a positive diagnosis of atopic dermatitis from a postal application of the United Kingdom diagnostic criteria for atopic dermatitis (45, 46). The primary exposures of interest were

maternal dietary antioxidant intakes (vitamin E, vitamin C, β -carotene, selenium, magnesium, manganese, copper, and zinc) and plasma concentrations of α -tocopherol, ascorbate, and β -carotene. The vitamins and intakes from supplements were added to give total nutrient intake and were energy adjusted and divided into fifths. For the outcome "wheeze in the absence of a cold," the nutrient data were divided into thirds due to the smaller number of cases present. Univariate associations between dependent variables and the independent variables were assessed with Mantel-Haenszel odds ratios, multivariate analysis being performed by logistic regression with adjustment for covariates. Multivariate analyses were performed separately for each of the follow-up time points (6 months, 12 months, and 24 months) because we anticipated that associations between maternal antioxidant intake during pregnancy and childhood wheeze and eczema outcomes were more likely to become apparent as the children grew up. Two subgroups of children were assessed; children who wheezed without a cold as their specific wheeze may be more relevant to asthma, and children of atopic mothers (one or more positive skin prick tests) as they are at a greater risk of developing atopic disease. Alternative measures of socioeconomic status (maternal social class, age of leaving full-time education, highest qualification attained, and deprivation index based on area of residence) were included separately into regression models.

In further analyses, for children with data at the three sampling points, parentally reported wheeze for the three time periods 0–6, 7–12, and 13–24 months were combined into a single dependent variable; no wheeze reported during any time period, wheeze reported during one time period and wheeze reported during two or more time periods. Eczema outcomes were also similarly categorized. The dependent variable combining outcomes during the first two years was analyzed using multinomial logistic regression with adjustment for the covariates, sex, maternal age, paternal social class, maternal atopy, maternal smoking, other children in home, antibiotic use, and maternal vitamin E or C intake. All analyses were done using SPSS v10.1 (SPSS Inc., Chicago, IL).

RESULTS

Between April 1998 and December 1999, 1,924 singletons were born to the 2,000 women. Thirty-four women gave birth to twins and were excluded from the study, and 42 women lost their babies through miscarriage, intrauterine death, stillbirth, or neonatal death. Follow-up of the original cohort was limited to the 1,924 singleton births.

The FFQ was satisfactorily completed in late pregnancy by 1,751 (87.6%) of the mothers. The response rates to the health questionnaire at 6, 12, and 24 months were 1,637 (85.1%), 1,512 (78.6%), and 1,374 (71.4%), respectively. Complete data sets from all three questionnaires were available for 1,300 (67.6%). Some families moved away from the area and were lost to follow-up, while others failed to respond (perhaps because their lifestyles became more demanding after returning to work and/or the birth of another child). When compared with the mothers who failed to complete the 2-year questionnaire, respondent mothers were older, more likely to be from a nonmanual social class, more likely to have had further education, more likely to have completed the FFQ, and less likely to smoke. However, response appeared to be unrelated to atopic status or parity (Table 1). Similar differences were found between responders and nonresponders at 6 and 12 months (data not shown).

Wheeze

The period prevalence of wheeze during the 2-year follow up is detailed in Table 2. There were no consistent statistically significant associations between wheezing symptoms in the first 2 years of life and total maternal intakes of β -carotene, selenium, magnesium, manganese, copper, and zinc. For the symptom of "ever wheeze" there were no consistent significant associations with maternal total or dietary intake of vitamin E (Table 3 and Tables

TABLE 1. COMPARISON OF MOTHERS COMPLETING OR FAILING TO COMPLETE 2-YEAR FOLLOW-UP QUESTIONNAIRE

Maternal Characteristic	Respondent (n = 1,374)	Nonrespondent (n = 550)	p Value
Age at booking, yr mean (SD)	29.8 (5.1)	26.9 (6.0)	< 0.001
Partner's social class % Nonmanual	64.8	53.4	< 0.001
Educational status % Further education	57.6	40.5	< 0.001
Atopic mother, % positive	36.8	33.3	0.138
Current smoker % When recruited	23.4	43.8	< 0.001
% At 34 wk gestation	15.7	30.7	< 0.001
First pregnancy, %	49.2	49.7	0.868
Completed Food Frequency Questionnaire, %	97.1	70.0	< 0.001

E1 and E2 in the online supplement). In the second year of life, 77 children were reported to have wheezed in the absence of a "cold" and there were significant negative associations with maternal total vitamin E intake that persisted after adjustment for confounding variables including total vitamin C intake (Table 3). Total maternal vitamin C intake during pregnancy was not statistically significantly associated with wheezing during the first year of life (Table E3). However, in the second year of life total vitamin C intake was positively associated with wheezing after adjustment for potential confounding variables including total vitamin E intake, *p* for trend 0.010 (Table 4).

There were no consistent significant associations found between wheeze and nutrient intake in the children of atopic mothers group (Tables E4 and E5). No further associations were noted between maternal and cord blood α -tocopherol, ascorbate, and β -carotene levels and wheezing symptoms or eczema in the first year of life. Wheeze (but not wheeze in the absence of a "cold") in the second year of life was positively associated with measured maternal plasma ascorbate at recruitment (odds ratio [OR] 1.18, 95% CI 1.00–1.40, *p* = 0.052) after adjustment for confounding variables including gestational age at recruitment.

Modeling of all available wheeze data from the 6-, 12-, and 24-month questionnaires demonstrated a significant positive association between total maternal vitamin C intake and the likeli-

hood of wheezing being reported on two or more occasions during the first 2 years of life (OR 1.187, 95% CI 1.031–1.367, *p* = 0.017). For wheezing in the absence of a "cold", although there were negative associations with total maternal vitamin E intake, these did not achieve statistical significance, probably because of the very small numbers of children who had wheezed during two or more time periods for each of the thirds of vitamin E intake.

Eczema

The period prevalence of eczema during the 2-year follow up is detailed in Table 2. There were no consistent statistically significant associations between eczema in the first 2 years of life and total maternal intakes of vitamin E, vitamin C, β -carotene, selenium, magnesium, manganese, copper, and zinc (Tables 5, 6, E2, and E3). There was a weak positive association between maternal total vitamin C intake during pregnancy and eczema in the second year of life (Table 6). Restriction of the analysis to the children predisposed to eczema by having an atopic mother demonstrated a statistically significant negative association between maternal total vitamin E intake during pregnancy and eczema in the second year of life (Table 5). This association was statistically significant after adjustment for confounding variables including total maternal vitamin C intake. There were no associations between maternal total vitamin C intake by atopic mothers during pregnancy and atopic eczema in their children during the first two years of life (Table 6). No associations were noted between dietary intakes of vitamins E or C and eczema in the second year of life (Tables E1, E2, and E3).

The consumption of more than one portion of fruit a day was positively associated with eczema in the second year of life, both before (OR 1.72, 95% CI 1.22–2.43, *p* = 0.002) and after adjustment (OR 1.67, 95% CI 1.16–2.40, *p* = 0.006) for potential confounding variables. Maternal consumption of fruit juices or vegetables was not significantly associated with wheeze or eczema in the first 2 years of life. No associations between fruit or vegetables and plasma levels were found.

Modeling of all available eczema data from the 6-, 12-, and 24-month questionnaires demonstrated that in the first 2 years of life there were positive associations between maternal vitamin C intake and the likelihood of parental reporting of eczema during one time period (OR 1.143, 95% CI 1.023–1.278, *p* = 0.018) and with eczema being reported during two or more time periods (OR 1.121, 95% CI 1.002–1.254, *p* = 0.045). There was also a negative association between maternal vitamin E intake and the likelihood of two or more reports of eczema during the first 2 years in children with atopic mothers (OR 0.831, 95% CI 0.700–0.985, *p* = 0.033).

TABLE 2. PERIOD PREVALENCE OF WHEEZE AND ECZEMA IN THE FIRST 2 YEARS OF LIFE

	0–6 mo n (%)	7–12 mo n (%)	13–24 mo n (%)
Wheeze	313 (19.1)	233 (15.4)	210 (15.3)
Wheeze in absence of "cold"	98 (6.0)	88 (5.8)	77 (5.6)
Wheeze at night	165 (10.1)	151 (10.0)	153 (11.1)
Wheeze, children with atopic mothers	137 (23.2)	101 (18.5)	98 (19.4)
Wheeze absence of a "cold", atopic mothers	39 (6.6)	45 (8.2)	40 (7.9)
Atopic dermatitis (UK Diagnostic Criteria definition) (45)	327 (17.9)	249 (16.5)	310 (22.6)
Atopic dermatitis (UK Diagnostic Criteria definition) (45) and/or doctor-diagnosed eczema	362 (22.4)	290 (21.6)	358 (26.1)
Atopic dermatitis (UK Diagnostic Criteria definition) (45) and/or doctor-diagnosed eczema in children with atopic mothers	159 (27.0)	146 (26.7)	160 (31.6)

DISCUSSION

This study prospectively investigated whether maternal antioxidant intake during pregnancy is associated with the development of respiratory symptoms and atopic disease in early childhood. The study population were very similar in demographic and obstetric characteristics to the local obstetric population, but there was some loss to follow-up with time, with the response rate at 24 months being lower among mothers with lower educational attainment and lower socioeconomic status and those who smoked in pregnancy. Although we found no significant difference in maternal vitamin C and vitamin E intakes between mothers who responded and those who did not respond, plasma levels of vitamin C and vitamin E were higher in the responders than the nonresponders (data not shown). This is consistent with other studies that have found higher blood levels of these vitamins, particularly vitamin C, in adults from higher socioeconomic

TABLE 3. ASSOCIATIONS BETWEEN TOTAL MATERNAL VITAMIN E INTAKE DURING PREGNANCY AND WHEEZE OR WHEEZE IN THE ABSENCE OF A "COLD" IN THE SECOND YEAR OF LIFE

	13–24 mo		
	Unadjusted OR (95% CI)	Adjusted* OR (95% CI)	Adjusted [§] OR [including total vitamin C] (95% CI)
Ever wheeze in total cohort—cases 15%			
1st fifth (lowest)	1.00	1.00	1.00
2nd fifth	0.78	0.73	0.55
	(0.49–1.24)	(0.44–1.21)	(0.30–1.03)
3rd fifth	0.69	0.70	0.54
	(0.43–1.11)	(0.42–1.44)	(0.30–1.00)
4th fifth	0.70	0.71	0.57
	(0.44–1.12)	(0.43–1.18)	(0.30–1.09)
5th fifth (highest)	0.70	0.79	0.53
	(0.43–1.12)	(0.47–1.31)	(0.27–1.01)
p Value (trend)	0.173	0.363	0.183
Wheeze in the absence of a "cold"—cases 6%			
1st third (lowest)	1.00	1.00	1.00
2nd third	0.59	0.61	0.57
	(0.34–1.02)	(0.35–1.07)	(0.32–1.02)
3rd third (highest)	0.45 [†]	0.47 [†]	0.49 [‡]
	(0.25–0.82)	(0.25–0.86)	(0.26–0.93)
p Value (trend)	0.006 [†]	0.010 [†]	0.009 [†]

*OR adjusted for sex, maternal age, paternal social class, maternal atopy, maternal smoking, other children in the home, and antibiotic use.

[†] p < 0.01.

[‡] p < 0.05.

[§] OR adjusted for the above with the addition of vitamin C.

groups (47). Despite this, we consider it unlikely that response bias could account for the observed associations between maternal vitamin intake and childhood wheeze, as among the respondents there was no evidence for any association between socioeconomic status and childhood wheeze. However, eczema was significantly

more common in children from nonmanual families, so we cannot rule out the possibility that lower response from mothers from lower socioeconomic groups, who may have had lower vitamin E intake and lower prevalence of eczema in their children, could have contributed to the inverse association between

TABLE 4. ASSOCIATIONS BETWEEN TOTAL MATERNAL VITAMIN C INTAKE DURING PREGNANCY AND WHEEZE OR WHEEZE IN THE ABSENCE OF A "COLD" IN THE SECOND YEAR OF LIFE

	13–24 mo		
	Unadjusted OR (95% CI)	Adjusted [‡] OR (95% CI)	Adjusted [§] OR [including total vitamin E] (95% CI)
Ever wheeze in total cohort—cases 15%			
1st fifth (lowest)	1.00	1.00	1.00
2nd fifth	1.42	1.79 [†]	2.34 [†]
	(0.84–2.41)	(1.00–3.19)	(1.15–4.35)
3rd fifth	1.96*	2.50*	2.63*
	(1.18–3.26)	(1.45–4.39)	(1.38–5.02)
4th fifth	1.25	1.52	1.83
	(0.73–2.14)	(0.83–2.77)	(0.91–3.66)
5th fifth (highest)	1.69 [†]	2.25*	3.00*
	(1.01–2.83)	(1.26–4.02)	(1.47–6.12)
p Value (trend)	0.114	0.034 [†]	0.010 [†]
Wheeze in the absence of a "cold"—cases 6%			
1st third (lowest)	1.00	1.00	1.00
2nd third	1.64	1.94 [†]	2.03 [†]
	(0.95–2.85)	(1.09–3.47)	(1.14–3.63)
3rd third (highest)	0.81	0.93	1.15
	(0.43–1.53)	(0.48–1.82)	(0.57–2.31)
p Value (trend)	0.560	0.288	0.065

*p < 0.01.

[†] p < 0.05.

[‡] OR adjusted for sex, maternal age, paternal social class, maternal atopy, maternal smoking, other children in the home, and antibiotic use.

[§] OR adjusted for the above with the addition of vitamin E.

TABLE 5. ASSOCIATIONS BETWEEN TOTAL MATERNAL VITAMIN E INTAKE DURING PREGNANCY AND ECZEMA IN THE "TOTAL COHORT" AND ALSO IN THE "CHILDREN OF ATOPIC MOTHERS" GROUPS IN THE SECOND YEAR OF LIFE

	13–24 mo		
	Unadjusted OR (95% CI)	Adjusted [†] OR (95% CI)	Adjusted [§] OR [including total vitamin C] (95% CI)
Eczema in total cohort – cases 26%			
1st fifth (lowest)	1.00	1.00	1.00
2nd fifth	1.12 (0.76–1.65)	1.04 (0.69–1.56)	1.00 (0.67–1.51)
3rd fifth	0.91 (0.62–1.35)	0.83 (0.55–1.24)	0.78 (0.51–1.18)
4th fifth	1.01 (0.68–1.48)	0.91 (0.60–1.36)	0.86 (0.57–1.30)
5th fifth (highest)	0.92 (0.62–1.37)	0.84 (0.55–1.28)	0.72 (0.46–1.13)
p Value (trend)	0.540	0.292	0.109
Eczema in "children of atopic mothers group"—cases 32%			
1st fifth (lowest)	1.00	1.00	1.00
2nd fifth	0.71 (0.39–1.28)	0.77 (0.41–1.44)	0.50 [†] (0.27–0.94)
3rd fifth	0.43* (0.23–0.78)	0.44 [†] (0.23–0.83)	0.39* (0.20–0.73)
4th fifth	0.93 (0.53–1.66)	0.83 (0.45–1.51)	0.50 [†] (0.26–0.94)
5th fifth (highest)	0.44 [†] (0.23–0.84)	0.41 [†] (0.21–0.80)	0.42 [†] (0.22–0.82)
p Value (trend)	0.070	0.024 [†]	0.016 [†]

* $p < 0.01$.† $p < 0.05$.

‡ OR adjusted for sex, maternal age, paternal social class, maternal atopy, maternal smoking, other children in the home, and antibiotic use.

§ OR adjusted for the above with the addition of vitamin C.

maternal vitamin E intake and eczema observed here. It is unlikely that mothers altered their diet because of participation in the study, because they were unaware of the dietary focus of the study until they received the FFQ. 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 (48). For these reasons the moderate and weak associations reported here between maternal antioxidant vitamin intake and early childhood wheeze and eczema are likely to be underestimates of the true association.

One of the weaknesses of this study is the reliance on postal questionnaires to follow the children up. Ideally each child would have been assessed by a pediatrician with an expertise in eliciting respiratory symptoms and diagnosing eczema, but this was not a practical option. The questionnaire was based on the established ISAAC format (44); the questions asked have been used in postal formats in this age group, and the replies have been shown to be reproducible (49–51). Identification of eczema was assessed by a report of doctor-diagnosed eczema and the responses to a validated postal application of the UK Working Party's Diagnostic Criteria for Atopic Dermatitis (45, 46). Although there are limited data validating the use of these diagnostic criteria in this age group, it is likely that misclassification of eczema would attenuate any associations rather than generate spurious ones.

We have demonstrated moderately strong negative associations between the total maternal intake of vitamin E during pregnancy and early childhood wheeze in the absence of a cold in the second year of life, and atopic eczema in the children

of atopic mothers. The demonstration of associations between maternal dietary vitamin E intake and outcomes in the second year of life is consistent with the hypothesis that maternal antioxidant intake during pregnancy modulates susceptibility to asthma and atopic disease (8). As the child grows up further relevant environmental exposures (e.g., childhood diet, allergen exposure) are necessary before the onset of asthma and atopic disease. A previous study of maternal dietary intake during pregnancy reported no associations between vitamin E and wheezing, asthma, or eczema in 5- to 6-year-old children (52). In previous studies of adults, positive associations have been demonstrated between dietary vitamin E, plasma α -tocopherol, and ventilatory function (18, 19, 53, 54). However, these findings were mostly in older subjects, some of whom had COPD rather than asthma. Studies possibly more relevant to asthma and atopy in adults have demonstrated negative associations between dietary vitamin E intake and adult-onset wheeze (24), the incidence of physician-diagnosed asthma over a 10-year period (22), serum IgE concentrations, and frequency of allergen sensitization (31). In a study of 12-year-old children, dietary intake of vitamin E was negatively associated with asthma and wheezing (28). A previous study of this population suggests that maternal total vitamin E intake during pregnancy influences the developing fetal immune system. In a subgroup of 223 children we have demonstrated associations between maternal total vitamin E intake during pregnancy and T-helper cell responses at birth after stimulation by the allergens timothy grass and house dust mite (36). Published studies demonstrating associations between childhood atopic disease and cord blood T-helper cell responses suggest that the association between maternal vitamin E intake during pregnancy and cord blood T-helper cell responses are likely

TABLE 6. ASSOCIATIONS BETWEEN TOTAL MATERNAL VITAMIN C DURING PREGNANCY AND ECZEMA IN THE "TOTAL COHORT" AND ALSO IN THE "CHILDREN OF ATOPIC MOTHERS" GROUPS IN THE SECOND YEAR OF LIFE

	13–24 mo		
	Unadjusted OR (95% CI)	Adjusted [‡] OR (95% CI)	Adjusted [§] OR [including total vitamin E] (95% CI)
Maternal total vitamin C intake (eczema in total cohort—cases 26%)			
1st fifth (lowest)	1.00	1.00	1.00
2nd fifth	1.04	0.94	0.95
	(0.68–1.58)	(0.60–1.47)	(0.61–1.49)
3rd fifth	1.75*	1.78†	1.81*
	(1.18–2.61)	(1.17–2.70)	(1.19–2.75)
4th fifth	1.14	1.05	1.10
	(0.75–1.73)	(0.68–1.64)	(0.71–1.72)
5th fifth (highest)	1.52†	1.41	1.56
	(1.02–2.27)	(0.91–2.18)	(0.99–2.45)
p Value (trend)	0.044†	0.115	0.048†
Maternal total vitamin C intake (eczema in "children of atopic mothers group"—cases 32%)			
1st fifth (lowest)	1.00	1.00	1.00
2nd fifth	0.95	0.78	0.83
	(0.53–1.79)	(0.40–1.55)	(0.41–1.65)
3rd fifth	1.66	1.56	1.58
	(0.91–3.04)	(0.82–2.98)	(0.82–3.04)
4th fifth	0.92	0.82	0.95
	(0.49–1.72)	(0.42–1.61)	(0.47–1.89)
5th fifth (highest)	1.49	1.37	1.99
	(0.81–2.75)	(0.70–2.65)	(0.97–4.06)
p Value (trend)	0.275	0.395	0.105

*p < 0.01.

†p < 0.05.

[‡] OR adjusted for sex, maternal age, paternal social class, maternal atopy, maternal smoking, other children in the home, and antibiotic use.[§] OR adjusted for the above with the addition of vitamin E.

to be translated into associations between maternal vitamin E intake and childhood atopic disease (55–58). The associations between maternal total vitamin E and wheeze in the absence of a "cold" and atopic eczema in the children of atopic mothers supports the notion that the consumption of foods containing vitamin E during pregnancy influences the fetal immune system and the likelihood of early childhood asthma and atopic disease rather than modulating embryonic pulmonary development or infant immune responses to infection. Relative vitamin E deficiency has been associated with Th2-biased immune responses (12, 31), with fetuses and infants possibly being particularly sensitive (11). If the earliest encounters between Th-cells and allergens during fetal and neonatal life are biased toward the Th2 phenotype by a maternal diet deficient in vitamin E during pregnancy, it is likely that subsequent Th-cell differentiation will be modified by other environmental exposures that have the potential to either consolidate the Th2 phenotype further, or promote Th-cell differentiation toward the Th0 or Th1 phenotypes (59). Further follow-up of this cohort will establish whether the associations demonstrated with wheeze and eczema in the second year of life translate into associations between maternal dietary vitamin E intake and asthma and atopy as children grow older. Although we adjusted for variables linked to the "hygiene" hypothesis (7)—namely, number of older children, number of children in the house, and use of antibiotics by the infant—we cannot eliminate the possibility that the observed associations between vitamin E intake and wheeze and eczema outcomes are a consequence of residual confounding by factors associated with a higher socioeconomic status and a healthy lifestyle.

The positive association between maternal vitamin C intake and wheeze in children in the second year was an unexpected result, as virtually all previous studies that have investigated associations between dietary vitamin C, fruit, asthma, and respiratory symptoms have reported beneficial associations. In adults dietary vitamin C intake and blood ascorbate levels have been positively associated with ventilatory function (14–17, 54, 60). In adults negative associations have been demonstrated between dietary vitamin C intake and respiratory symptoms (60), methacholine bronchial hyperreactivity (23), and adult onset wheeze (24). Studies of fruit intake have demonstrated positive associations with ventilatory function in adults (18, 19, 61) and children (13, 20). Dietary vitamin C and fruit intake have been negatively associated with wheezing symptoms in adults (62) and children (21, 30, 63) and with adult asthma (27). In contrast, in the present study we have demonstrated positive associations between maternal consumption of foods rich in dietary vitamin C and wheezing symptoms and eczema in the second year of early childhood. Furthermore, there was a weak positive association between maternal plasma ascorbate levels and early childhood wheeze in the second year, and a strong positive association between maternal fruit consumption and atopic eczema in the second year of life. Similar trends were found between dietary intakes of vitamin C and E and the outcome variables of interest (Table E2). These paradoxical findings were surprising, but cannot easily be ignored. A positive association between baseline total vitamin C intake (but not dietary vitamin C intake) and the 10-year incidence of asthma was also reported in the U.S. Nurses Health Study (22). It was suggested that this association was the

result of reverse causation, the nurses who had subclinical asthma having appeared to increase their use of vitamin C supplements. In the present study the pregnant women may have paid greater attention to their diet and used vitamin supplements to improve their own health and that of their child, but this was not widespread as only 10% took supplements containing vitamin C. The associations with vitamin C were not a consequence of confounding by vitamin E as these vitamins are found in differing food groups and the effects persisted in models which included both nutrients. One possible explanation for a positive association between vitamin C intake and wheeze is that at high concentrations vitamin C can have a prooxidant effect, though it is not clear whether this occurs *in vivo* (64) and if so, whether prenatal exposure could influence risk of wheeze in childhood. Another possibility is that there are other naturally occurring or added constituents of foods containing vitamin C which can promote the development of wheeze in early life. Sulfites added as preservative to fruit drinks have been shown to cause wheeze in susceptible individuals (65), though again it is not clear whether prenatal exposure could influence postnatal risk. The alternative explanation is that the associations with vitamin C could be due to residual confounding by unmeasured behavioral difference between those with high and low intakes. Although fruit intake is generally associated with better rather than worse self-reported health in adults (66), it is possible that mothers with high vitamin C intakes were more likely to notice or report wheeze in their children, leading to a spurious positive association between vitamin C intakes and wheeze. In addition, the positive association between maternal vitamin C intake and wheeze was not seen with wheeze in the absence of a cold, which may be a better indicator of wheeze with an asthmatic etiology as opposed to viral associated wheeze. Further evidence that the observed association between vitamin C and wheeze may not be a true biological effect is provided by the finding that in a subsample of 223 of these children, cord blood responses to *in vitro* stimulation with allergens were inversely associated with maternal vitamin E intake but showed no association with vitamin C intake (36).

One study in adults has shown selenium intake in adults to be negatively associated with asthma (27). Another study has shown that umbilical cord selenium concentration is negatively associated with persistent wheeze at 0 to 6 months and at 30 to 42 months (52). In the present study no associations were found between maternal dietary intake of selenium and wheeze or eczema in the first 2 years of life. It should be noted that the FFQ was less effective in quantifying selenium intake, with the correlation coefficient for selenium being weaker than that of vitamins E and C. It is known that dietary selenium is relatively unreliable, as our main source of selenium is cereals, and the soil content in which they are grown differs in the level of selenium in various areas. To address this problem, further analysis is underway in the same population to assess the selenium content within the mother's blood and cord blood and to determine whether there are associations between selenium levels and wheeze and eczema in the children.

There are two important considerations when interpreting the finding of this study. In common with most observational studies of diet it was necessary to perform multiple comparisons with implications for chance significant associations. However, our previous work has highlighted vitamin C and E, and these were our principal nutrients of interest. The associations with vitamin E are consistent within this study and with other published studies. In contrast, the adverse associations with vitamin C, although consistent within this study, are inconsistent with published studies that have either demonstrated no or beneficial associations with vitamin C. It is highly likely that the associations with vitamin C demonstrated in this study are a consequence of

residual confounding by factors associated with socioeconomic status and/or healthy lifestyle. Although the associations reported here relate to maternal diet during pregnancy, it is likely that maternal and childhood diet will be positively associated. Further follow-up of these children will be used to assess whether the associations reported here persist into later childhood and to explore the possibility that any associations may be modified by childhood diet. For these reasons confirmation of these results in other studies and clarification of the role of childhood diet are needed before any recommendations can be made to pregnant women.

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