

Original article

Dietary antioxidant intake, allergic sensitization and allergic diseases in young children

Background: Allergic diseases have risen in prevalence over recent decades.

The aetiology remains unclear but is likely to be a result of changing lifestyle and/or environment. A reduction in antioxidant intake, consequent to reduced intake of fresh fruits and vegetables, has been suggested as a possible cause.

Objective: To investigate whether dietary antioxidant intake at age 5 was related to atopy at 5 and 8 years of age amongst children in an unselected birth cohort.

Methods: Children were followed from birth. Parents completed a validated respiratory questionnaire and children were skin prick tested at 5 and 8 years of age. Serum IgE levels were measured at age 5. At age 5, antioxidant intake was assessed using a semi-quantitative food frequency questionnaire (FFQ). A nutrient analysis program computed nutrient intake, and frequency counts of foods high in the antioxidant vitamins A, C and E were assessed.

Results: Eight hundred and sixty-one children completed both the respiratory and FFQ. Beta-carotene intake was associated with reduced risk of allergic sensitization at age 5 [0.80 (0.68–0.93)] and 8 [0.81 (0.70–0.94)]. In addition, beta-carotene intake was negatively associated with total IgE levels ($P = 0.002$). Vitamin E intake was associated with an increased risk of allergic sensitization [1.19 (1.02–1.39)], only at age 5. There was no association between antioxidant intakes and wheeze or eczema.

Conclusion: Increased beta-carotene intake was associated with a reduced risk of allergic sensitization and lower IgE levels, in 5- and 8-year-old children. Dietary antioxidants may play a role in the development of allergic sensitization.

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Key words: allergic sensitization; antioxidants; children; diet; wheeze.

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Accepted for publication 25 March 2009

The prevalence of atopy and allergic diseases has risen markedly over the last few decades (1). The cause of this increase is unlikely to be as a result of genetic shifts and is probably attributable to changes in lifestyle and/or the environment. Amongst many other environmental factors, variations in diet have been proposed as a possible cause of the increase in allergies (e.g. a reduction in the intake of antioxidant vitamins C, E and beta-carotene, consequent to a reduction in intake of fresh green vegetables) (2). Although there are a number of studies which investigated the relationship between dietary antioxidant intake and allergic diseases in adults, there is a paucity of data in young children.

The majority of studies investigating the role of diet in early childhood have concentrated on individual foods (3–5), and reported a protective role for fruit and vegetable intake. This protective effect has been attributed to vitamin C intake, but most of the studies have not attempted to assess the total antioxidant intake. To our knowledge, only Hijazi et al. (6) have undertaken total nutrient analysis in young children and related it to atopy and wheeze. These results were contrary to the findings

reported by studies assessing just fruit and vegetable intake, and showed a protective effect of vegetables and vitamin E intake, but not of vitamin C and fruit intake, on current wheeze.

Studies which investigated the effect of antioxidant supplementation on respiratory or allergic diseases in adults have mostly shown no beneficial effect of supplements (7–10). This discrepancy between epidemiological data relating to fruit and vegetable intake compared with intervention studies using antioxidant supplements may indicate the importance of the whole food, a related nutrient in the food or related dietary patterns, rather than individual items (e.g. vitamin C only).

Several studies have demonstrated that dietary habits (11) and nutrient intakes (12, 13) of children track from the age of 3 years to age 5. We hypothesized that the antioxidant intake assessed at the age of 5 years would be representative of the intake throughout early childhood. Within the context of a large population-based birth cohort, we aimed to assess whether dietary antioxidant intake measured at the age of 5 years is related to allergic sensitization and the presence of

allergic diseases at 5 and 8 years of age. In an attempt to distinguish the effects of food *vs* nutrient, we assessed both total nutrient intake, and also considered foods that are high in antioxidants.

Methods

Study population

The Manchester Asthma and Allergy Study (MAAS) is an unselected population-based birth cohort and is described in detail elsewhere (14, 15). Participants were recruited prenatally, and followed prospectively attending review clinics at ages 3, 5 and 8 years (± 4 weeks). The study is registered as ISRCTN72673620 and approved by the Local Research Ethics Committee. Written Informed consent was obtained from all parents.

Definition of outcomes

A validated respiratory questionnaire was interviewer-administered to collect information on parentally reported symptoms.

Wheeze. According to parentally reported history of wheeze, *current wheeze* at age 5 and 8 was defined as a positive response to the question 'Has your child had wheezing or whistling in the chest in the last 12 months?' Based on prospectively collected data, children were assigned to the following wheeze phenotypes.

No wheezing. No wheeze ever during the first 8 years of life.

Transient early wheezing. Wheezing during the first 3 years, no wheezing after the age of 3 years.

Late-onset wheezing. Wheeze onset after the child's fourth birthday.

Persistent wheezing. Wheezing at all time points (3, 5 and 8 years).

Intermittent wheezing. Wheezing at only one or two time points.

Eczema. This was defined as eczema present at the age of 5 or at the age of 8 as diagnosed during the follow-up at ages 5 and 8 at the follow-up clinic.

Allergic sensitization. This was ascertained at 5 and 8 years of age by skin prick testing (mite, cat, dog, grasses, milk and egg; Bayer, Elkhart, IN, USA); sensitization was defined as wheal diameter of at least 3 mm greater than the negative control to at least one of the standard allergens. In addition, a blood sample was taken to measure total IgE antibodies at the age of 5 years using ImmunoCapTM (Phadia AB, Uppsala, Sweden).

Definition of exposures

Dietary intake questionnaire. A semi-quantitative food frequency questionnaire (FFQ; DietQ, Tinuviel Software, Anglesey, UK) was completed by parents regarding their child's usual dietary intake during the review at the age of 5 years. The DietQ is composed of questions about the consumption of 100 food and drink items. It asks responders to rate how often each food is eaten based on a 9-level scale, from 'rarely to never', 'fortnightly' to 'seven times per week'.

Nutrient intake. It was calculated using DietQ, a nutrient intake analysis program (Tinuviel Software), and the Food Standards Agency's Composition of Foods, 6th Edition (16).

Frequency count. Foods high in vitamin C, E and A (retinol and beta-carotene) were identified using data from the Food Standards Agency's Composition of Foods, 6th Edition (16). The frequency with which each such identified food was consumed each week was calculated to give a frequency count.

Statistical analysis

Analysis was carried out using SPSS version 15 (SPSS Inc., Chicago, IL, USA). We analysed demographic and nutrient data using appropriate parametric tests wherever data or log-transformed data were normally distributed. Nutrient intakes were adjusted for energy using the calorie-adjusted method and a constant was added. The constant was the intake of the nutrient at mean calorie intake (17). Pearson's correlation was used to test the relationship between the two methods of assessing nutrient intake. Logistic regression analysis was used to analyse nutrient data when presented in quartiles and results are expressed as odds ratios (OR) with 95% confidence intervals (95% CI). To adjust for multiple testing, we only accepted *P*-values of ≤ 0.01 as significant and a stepwise method was used. For sensitization, gender and parental sensitization were adjusted for. For current wheeze, gender, parental sensitization, parental asthma at recruitment, parental smoking at follow-up and sensitization of child were used as covariates. For current eczema, parental sensitization and sensitization of the child were adjusted for. In addition, socioeconomic status and Z-scores for body mass index were added to the models. Further analysis for continuous outcome variables was carried out using ANOVA. Results are expressed as geometric means (GMs).

Results

A total of 1211 parents were recruited during pregnancy. Of the resulting 1085 healthy full-term deliveries, 128 children were prenatally randomized to an environmental control group and excluded from this analysis; 957 children were followed in the observational cohort (14). Food frequency questionnaire data was available for 861 children (90%). Blood samples were taken at the age of 5 years from 496 (57%) of children for IgE measurement. There were no differences between children with blood samples and those without in terms of gender, socioeconomic status, parental atopic status, current wheeze and eczema ($P > 0.1$). Table 1 shows the characteristics of the study population.

A summary of mean nutrient intakes is presented in Table 2, along with reference nutrient intakes (RNIs). Mean intakes for beta-carotene were equivalent to the RNI in our cohort, whilst intakes of vitamin C were 3.5 times higher than the RNI. However, mean intakes of vitamin E were half of the recommended intake. Nutrient intakes and frequency counts and for all three antioxidants were significantly correlated ($P < 0.01$); however, correlates were relatively low, particularly for vitamins C

Table 1. Demographic data and patient characteristics

Characteristics	N	%
Male	474/861	55
Maternal atopy	458/846	54.1
Paternal atopy	495/844	58.6
Maternal smoking, age 5	150/843	17.8
Paternal smoking, age 5	204/843	24.2
Maternal smoking, age 8	129/805	16.0
Paternal smoking, age 8	166/790	21.0
Maternal asthma at recruitment	114/860	13.3
Paternal asthma at recruitment	54/860	6.3

Table 2. Dietary intake of antioxidant vitamins (computed nutritional intakes and frequency counts)

Variable	Mean	95% CI	Reference nutrient intake
Nutrient intake (computed)			
Beta-carotene (μg) (retinol equivalent)	2354.7 (392.5)	2276–2433	400*
Vitamin C (mg)	104.6†	101–108	30
Vitamin E (mg)	3.52‡	3.46–3.63	7‡
Frequency count			
Vitamin A	5.64	3.28	–
Vitamin C	10.5	5.37	–
Vitamin E	4.18	3	–

Frequency count – the number of food portions eaten per week that are high in the stated vitamin.

*Retinol equivalent.

†Geometric mean.

‡USA data (36).

and E (vitamin C: $r = 0.34$; vitamin E: $r = 0.34$) and higher for vitamin A/beta-carotene ($r = 0.71$).

Higher nutrient intakes of beta-carotene were significantly associated with a lower risk of sensitization at the age of 5 (Table 3) and 8 years (Table 4) both in the univariate analysis ($P = 0.002$) and following adjustment for confounders ($P = 0.004$). In contrast, higher nutrient intakes of vitamin E were significantly associated with a higher risk of sensitization ($P = 0.01$) at the age of 5 years (Table 3) with a similar nonsignificant trend at the age of 8 years ($P = 0.1$). However, the frequency counts for both vitamins A and E were not associated with sensitization. No association was seen with vitamin C intake (nutrient intake or frequency counts) and sensitization.

We found that beta-carotene intake was significantly negatively associated with total serum IgE levels at the age of 5 years (Fig. 1). Total serum IgE level (KU/l) amongst children whose beta-carotene intake was in the lowest quartile was approximately two times higher compared with children whose beta-carotene intake was in the highest quartile [GM (95% CI): lowest quartile 44.5 (32.7–60.3); highest quartile 23.4 (17.6–31.1); $P = 0.002$]. No relationship was seen between vitamin E intake and serum IgE levels ($P = 0.35$).

In the whole population, vitamins A, C and E intakes and frequency counts were not associated with current wheeze, wheeze phenotypes and current eczema (results not shown, available on request). Amongst atopic children at the age of 5 years, there was a trend towards higher vitamin C intakes reducing the risk of current wheeze, which failed to reach statistical significance [0.77

Table 3. Odds ratio (95% CI) for sensitization at the age of 5 years by SPT for quartiles of vitamins A, C and E nutrient and frequency intakes

Vitamins	Univariate analysis		Multivariate analysis†	
	Frequency count	Nutrient intake	Frequency count	Nutrient intake
A				
1st quartile	1	1	1	1
2nd quartile	0.7 (0.44–1.1)	0.73 (0.47–1.14)	0.65 (0.4–1.06)	0.75 (0.47–1.20)
3rd quartile	1.1 (0.69–1.66)	0.59 (0.38–0.92)*	1.08 (0.67–1.74)	0.59 (0.37–0.96)*
4th quartile	0.77 (0.49–1.21)	0.52 (0.32–0.81)*	0.8 (0.49–1.30)	0.52 (0.32–0.85)*
Linear trend	0.96 (0.84–1.11)	0.80 (0.7–0.92)*	0.98 (0.84–1.14)	0.80 (0.68–0.93)*
C				
1st quartile	1	1	1	1
2nd quartile	0.82 (0.52–1.31)	1.16 (0.73–1.84)	0.84 (0.5–1.4)	1.15 (0.70–1.90)
3rd quartile	1.04 (0.69–1.63)	1.26 (0.80–2.00)	1.18 (0.72–1.93)	1.41 (0.86–2.31)
4th quartile	1.17 (0.75–1.83)	1.13 (0.71–1.79)	1.46 (0.89–2.38)	1.23 (0.74–2.03)
Linear trend	1.08 (0.93–1.24)	1.04 (0.90–1.21)	1.16 (0.99–1.36)	1.08 (0.93–1.27)
E				
1st quartile	1	1	1	1
2nd quartile	0.64 (0.39–1.05)	1.37 (0.85–2.21)	0.79 (0.46–1.35)	1.33 (0.8–2.21)
3rd quartile	0.88 (0.57–1.37)	1.36 (0.85–2.19)	1.16 (0.72–1.88)	1.24 (0.75–2.07)
4th quartile	0.92 (0.57–1.46)	1.84 (1.15–2.94)*	1.14 (0.67–1.91)	1.78 (1.08–2.95)*
Linear trend	1.0 (0.86–1.17)	1.20 (1.04–1.39)*	1.08 (0.92–1.28)	1.19 (1.02–1.39)*

* $P < 0.01$.

†Multivariate analysis adjusted for gender, parental atopy, socioeconomic status and BMI SDS.

Table 4. Odds ratio (95% CI) for sensitization at the age of 8 years by SPT for quartiles of vitamins A, C and E nutrient and frequency intakes

Vitamins	Univariate analysis		Multivariate analysis†	
	Frequency count	Nutrient intake	Frequency count	Nutrient intake
A				
1st quartile	1	1	1	1
2nd quartile	1.29 (0.80–2.07)	0.72 (0.47–1.11)	0.97 (0.61–1.53)	0.68 (0.43–1.07)
3rd quartile	1.03 (0.70–1.53)	0.67 (0.44–1.04)	1.0 (0.62–1.60)	0.68 (0.43–1.07)
4th quartile	0.76 (0.84–1.18)	0.49 (0.31–0.77)*	0.85 (0.53–1.35)	0.49 (0.31–0.79)*
Linear trend	0.92 (0.81–1.06)	0.80 (0.70–0.93)*	0.95 (0.82–1.11)	0.81 (0.70–0.94)*
C				
1st quartile	1	1	1	1
2nd quartile	0.88 (0.56–1.38)	1.12 (0.72–1.77)	0.94 (0.59–1.51)	1.17 (0.73–1.86)
3rd quartile	0.96 (0.62–1.48)	1.37 (0.88–2.12)	1.01 (0.63–1.61)	1.54 (0.98–2.43)
4th quartile	0.89 (0.57–1.35)	1.09 (0.69–1.71)	1.05 (0.65–1.69)	1.21 (0.75–1.95)
Linear trend	0.97 (0.85–1.12)	1.05 (0.91–1.21)	1.02 (0.88–1.19)	1.09 (0.94–1.27)
E				
1st quartile	1	1	1	1
2nd quartile	0.75 (0.47–1.19)	1.12 (0.72–1.76)	0.84 (0.52–1.36)	1.05 (0.66–1.67)
3rd quartile	0.80 (0.52–1.23)	1.04 (0.66–1.63)	0.82 (0.52–1.30)	1.0 (0.63–1.61)
4th quartile	0.69 (0.43–1.09)	1.49 (0.96–2.32)	0.77 (0.47–1.27)	1.52 (0.96–2.4)
Linear trend	0.90 (0.78–1.04)	1.12 (0.97–1.20)	0.92 (0.79–1.08)	1.13 (0.98–1.37)

* $P < 0.01$.

†Multivariate analysis adjusted for gender, parental atopy, socioeconomic status and BMI SDS.

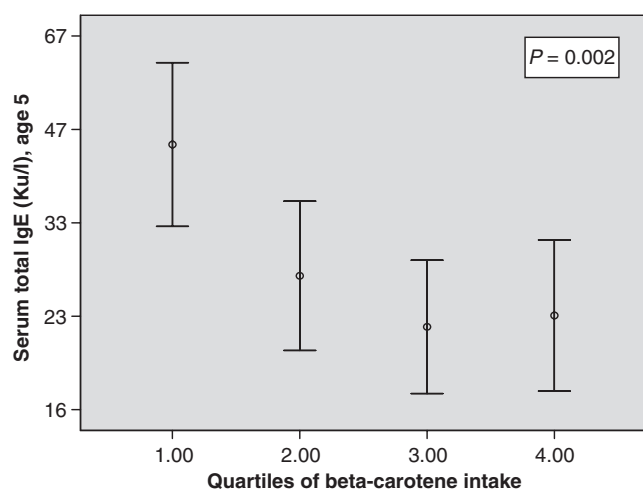


Figure 1. Error bar chart for total IgE (GM and 95% CI)* and quartiles of beta-carotene intake. *Unadjusted geometric means and 95% confidence intervals.

(0.58–1.01) $P = 0.06$]. At the age of 8 years, there was no relationship between current wheezing and vitamin C intake amongst atopic children. There were no relationships between current wheeze and vitamin E or beta-carotene intake at either time point.

Discussion

Principal findings

In this study, we investigated dietary intakes of antioxidants amongst children at the age of 5 years and their

relation to atopic sensitization and allergic disease at the age of 5 and 8 years respectively. We found higher beta-carotene intakes to be associated with a reduced risk of sensitization. In addition, beta-carotene intake was significantly negatively associated with serum total IgE concentrations. In contrast, higher vitamin E intakes were significantly associated with higher risk of sensitization, but only at the age of 5 years. We found no significant association between antioxidant intake and current eczema or current wheeze.

Interpretation

Beta-carotene is a precursor to vitamin A and has also been shown to have antioxidant properties. To our knowledge, there are no other studies demonstrating the negative association between beta-carotene intake and IgE levels and sensitization. Kompauer et al. (18) reported no significant relationship between serum levels of beta-carotene and allergic sensitization in adults, and analysis of the NHANES III data found similar results within children aged 6–16 years (19). However, several studies have reported a protective effect of beta-carotene intake on asthma (20, 21).

Beta-carotene has been shown to reduce the production of IgE in mouse models (22). Sato et al. (22) demonstrated an increase in Th1 cytokine production in the beta-carotene supplementation group and lower production of Th2 cytokines, thus influencing the immune response towards a Th1 type. In addition, beta-carotene has been found to modulate the production of prostaglandin E_2 by influencing the cyclooxygenase and

lipoygenase pathways (23, 24). As prostaglandin E₂ may promote development of the Th2 immune response, this may be a possible mechanism.

We observed a positive association between vitamin E intake and sensitization at the age of 5 but not at the age of 8 years. This finding is both unexpected and contrary to the previous reports by others (25, 26). Fogarty et al. (25) reported a protective effect of high vitamin E intake on allergic sensitization in adults, whilst Devereux et al. (26) reported no association between vitamin E and sensitization or asthma in 5-year-old children. One possible explanation for this inconsistency could be that our cohort had a very low intake of vitamin E, with the mean intake being half of the recommended dietary intake (RDI). In fact, only children in the highest quartile of vitamin E intake in our study achieved the RDI. It may be that children in the UK do have a very low intake of vitamin E, as a result of dietary advice given to parents of young children with regard to the avoidance of nuts and eggs (foods rich in vitamin E), because of the potential risks concerning allergy and aspiration. It is also possible that our questionnaire has under-estimated vitamin E intake in our cohort. However, examining adult intakes using our FFQ we have found similar vitamin E intakes compared with other adult studies in the UK (data not shown) (26).

Considering our very low intakes of vitamin E and also the fact that the association between vitamin E and sensitization was only observed at the age of 5 but not at the age of 8, and the results were not supported by an association with serum IgE, we believe that these results should be viewed with caution.

Our results also demonstrated a trend towards lower consumption of vitamin C amongst atopic wheezers as compared with atopic nonwheezers, but only at the age of 5. This has not been reported by other investigators. A number of studies which found a protective effect of fruit intake on wheeze in children have attributed this to the vitamin C content (3–5); our results do not support these findings.

Strengths and limitations

The dietary assessment method used in our study has been validated in adults (27–30). To our knowledge, there are no validated FFQs to assess the diet of British children. In addition, young children themselves are unable to report on their diet and parents and caregivers have to be relied upon. Parents can reliably inform of their child's dietary intake at home, but not for food and drink outside the home (31). Portion sizes were adjusted for use in children. Nutritional assessment relies on food composition tables and consideration has to be given to the accuracy of these as they provide the average nutrient content, which has been derived from a selection of each food item. Moreover, the variation of micronutrients in foods is greater than that of macronutrients and intake

may not relate to bioavailability because of changes during cooking and absorption.

In addition to the computed nutrient intakes, we looked at frequency of intake of foods high in vitamins A, C and E as a surrogate measure of intake, and demonstrated a reasonable agreement. Despite this agreement, the results for vitamins A and E were inconsistent between computed vitamin intake and frequency counts of intake. Vitamin E is contained in a large range of foods and the frequency method may not have captured this. For vitamin A, frequency counts, retinol and carotene were considered whilst the nutrient intake has concentrated on beta-carotene intake. This may indicate the value of using a FFQ opposed to assessing individual food intake.

The strength of this study is the sample size and the fact that the children are all of the same age at assessment; most other studies have looked at the intake of children within a broad age range (6, 32).

Early-life exposures are critically important for the development of allergic diseases (33), which emphasize the importance of studies investigating the antioxidant intakes in young children (34). Despite the importance of looking at the role of diet in the development of allergic disease in early childhood, there are few studies on this topic in children. For example, Forastiere et al. (3) examined the impact of foods high in vitamin C content in children aged 6–7 years, whilst Nja et al. (35) retrospectively assessed fruit and vegetable consumption in the first year of life and subsequent atopy at the age of 11 years. Our study adds to this small body of evidence of the impact on allergic disease of antioxidant intake in early childhood.

Conclusions

In recent years, there has been a change in dietary patterns, with a decrease in consumption of fresh green vegetables and potatoes which are the main contributors of vitamin C and beta-carotene. Our results indirectly support the hypothesis that a decrease in antioxidant consumption, in particular beta-carotene, may be associated with an increased risk of atopic sensitization.

Acknowledgments

The authors would like to thank the MAAS children and parents for their continued support. We would also like to acknowledge the dedication of the MAAS team.

Funding

Asthma UK Grant No 04/014 and Moulton Charitable Trust.

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