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# Serum vitamin E concentrations at 1 year and risk of atopy, atopic dermatitis, wheezing, and asthma in childhood: the PASTURE study

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## Keywords

asthma; atopy; children; vitamin E; wheeze.

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## Abstract

**Background:** Prospective studies investigating the role of serum vitamin E concentrations during early life in the development of childhood allergies and asthma are limited.

**Objective:** To study the associations between serum vitamin E concentrations at first year of life and longitudinal development of atopy, atopic dermatitis, wheeze, and asthma up to 6 years of age.

**Methods:** The setting was the PASTURE study, a multicenter prospective birth cohort study in five European rural settings. Children of 1133 mothers recruited during pregnancy were followed from birth with measurement of serum vitamin E levels at year 1 and repeated assessments of serum immunoglobulin E antibodies (year 1, 4.5, 6), atopic dermatitis, wheezing symptoms, and asthma (year 1, 1.5, 2, 3, 4, 5, 6).

**Results:** At 6 years of age, 66% and 82% of the original 1133 subjects underwent blood test for IgE and answered the questionnaire, respectively. We did not observe any statistically significant associations between serum vitamin E concentrations at year 1 and the endpoints, but borderline inverse associations between alpha tocopherol and wheezing without cold (OR 0.45, 95% CI 0.19–1.09) and any wheezing symptom (OR 0.52, 95% CI 0.27–1.02).

**Conclusions:** Serum vitamin E concentrations at year 1 were not associated with allergies or asthma by 6 years of age. While further prospective studies with repeated assessments of vitamin E during early life may clarify its putative role in the development of the diseases, it is also possible that the antioxidant hypothesis in the development of allergies and asthma does not hold.

According to the antioxidant hypothesis, the increase in the prevalence of allergies and asthma may be influenced by reduced intake of antioxidant-rich foods, particularly fruits and vegetables (1–6). Allergies and asthma are characterized by inflammatory processes that result in imbalance between T-helper cells 1 (Th1) and Th2, favoring Th2 dominance (2, 7, 8). The process of Th2 differentiation is among others in response to the presence of oxidative stress resulting from the activities of oxygen free radicals during which inflammatory mediators are also released (2, 7, 8). Antioxidants counter the effect of oxygen free radicals (2, 7, 8), hence, may confer protection against the development of allergies and asthma (3, 5, 6).

Vitamin E is a major antioxidant nutrient (3, 5, 6, 9), and, among its derivatives, alpha and gamma tocopherol may influence the development of allergies and asthma. Only a few studies have examined the link between children's vitamin E status and the risk of the diseases. Most existing studies have employed either case-control (10–13) or cross-sectional (14, 15) study designs, and the findings are inconsistent. Prospective studies investigating the influence of early life vitamin E serum concentrations on the development of allergies and asthma in childhood are lacking.

The Protection against Allergy – STUdy in Rural Environments study (PASTURE) is a prospective birth cohort investigating the early life events that may protect against the development of childhood allergic diseases and asthma in rural European settings (16). The current study presents the results of the associations between serum vitamin E concentrations at the age of 1 year and longitudinal development of atopy, atopic dermatitis, wheezing, and asthma up to the age of 6 years.

## Methods

### Study design and subjects

The details of the PASTURE study have been reported elsewhere (16). It is a multicenter prospective birth cohort study in rural areas of five European countries (Austria, Finland, France, Germany, and Switzerland). Pregnant women (excluding those <18 years, living on farms without livestock, with twin pregnancy, home births, premature deliveries, genetic disease in the offspring, lack of telephone connection, insufficient knowledge of the local language, intending to move away from study area) were contacted in the third trimester of pregnancy. Half of the women lived on farms with livestock.

Participating women completed questionnaires on lifestyle factors, respiratory and other diseases of parents and the child, agricultural exposures, and common potential confounders at the third trimester, at age 2, 12, 18, 24 months of the child, and annually up to the age of 6 years. At each follow-up, relevant exposures and the allergic and asthma endpoints were assessed by questionnaire and/or blood samples. Of the 2871 women contacted for the study, 62% were eligible, and of these 1133 participated originally in the study.

### Measurement of vitamin E and cholesterol

Serum alpha and gamma tocopherol were determined by an in-house micro HPLC method. To 50 µl serum, 0.4 ml of a 50% ethanolic solution containing ascorbic acid and BHT and 50 µl of internal standards tocol and echinenon were added. After mixing, the analytes were extracted with 1 ml hexane. A 0.8-ml hexane aliquot was evaporated under nitrogen and the residue dissolved in 100 µl of methanol. Alpha and gamma tocopherol were separated with an Inertsil ODS-3 column (2.1 × 100 mm, 3 µm, GL Sciences, Japan). The mobile phase was methanol, 0.3 ml/min and 5 µl were injected into the column, and the tocopherol was detected by fluorescence at 292/324. Peak height/internal standard ratios were compared to the ratios of reference plasma whose values were traceable to NIST certified serum standards, 968d (National Institute of Standardization and Technology, Gaithersburg). The mean precision between series for the alpha and gamma tocopherol was 5.7 and 6.5 CV%, respectively. The accuracy was ascertained by analyzing two external reference sera provided by Labquality (Finland). The mean bias for alpha tocopherol was –2.9%. Serum total cholesterol was measured on microplates with an enzymatic CHOD-PAP method using Abbott reagents (Abbott Laboratories, Abbott Park, IL, USA). The precision between series was 7.3 CV%.

### Assessment of the endpoints

At the ages of 1, 1.5, 2, 3, 4, 5, and 6 years, a questionnaire developed within PASTURE study group was used to assess the allergic and asthma status of the child. The questionnaire used questions from the International Study of Allergy and Asthma in Childhood (ISAAC), the Allergy and Endotoxin study (ALEX), the PARSIFAL study, the Asthma Multicenter Infants Cohort Study, and the American Thoracic Society (ATS) questionnaire. 'Atopic dermatitis' was defined as parental-reported doctor-diagnosed atopic dermatitis at least once during the follow-up. Asthma was defined as parental-reported doctor-diagnosed asthma and/or asthmatic (or obstructive) bronchitis at least twice during the follow-up. 'Wheezing without cold' was defined as parental-reported wheezing or whistling without having a cold in the past 12 months (past 10 months in the 1-year, past 6 months in the 1.5-year and 2-year questionnaires, and past 12 months in 3–6-year questionnaires) and 'any wheezing symptom' as parental-reported wheezing symptom.

At the ages of 1, 4.5, and 6 years, venous blood samples were obtained from the children and analyzed for specific immunoglobulin E (sIgE) to 19 common allergens, using the Allergy Screen Test Panel for Atopy (Mediawiss Analytic, Moers, Germany) (17). The 13 inhalative allergens tested *Dermatophagoides pteronyssinus*, *D. farinae*, alder, birch, European hazel, grass pollen mixture, rye, mugwort, plantain, cat, horse, and dog dander, and *Alternaria alternata*. The six food allergens were hen's egg, cow's milk, peanut, hazelnut, carrot, and wheat. Based on observed prevalence, atopic sensitization at the age of 1 year was defined as sIgE concentrations of 0.35 kU/l or greater, while it was defined

as sIgE concentrations 0.70 kU/l or greater at the ages of 4.5 and 6 years.

### Statistical analysis

Descriptive analyses were performed using simple frequencies, ANOVA, and the Student's *t*-test. Atopic sensitization and wheezing were analyzed with generalized estimating equations (GEE) to account for the repeated measurements of the endpoints, using the exchangeable correlation structure. The onset (incident) of atopic dermatitis and asthma was analyzed using discrete hazard model because the follow-up time was interval censored. Current asthma at 6 years (i.e., defined as incident asthma during follow-up plus wheeze or use of asthma medication during the 12 months preceding the age of 6 years) was analyzed using discrete hazard model. Because vitamin E was measured at the age of 1 year, to estimate the prospective associations between vitamin E and the risk of atopic dermatitis, wheezing, and asthma, we excluded the endpoints occurring during year 1. Two sets of models were fitted for all endpoints: The first model included center of study, cholesterol, and farming status (as well as time for the wheezing and sensitization endpoints) as covariates. Vitamin E is transported by lipoproteins, leading to an interaction between vitamin E levels and cholesterol concentrations in the blood. Thus, it is recommended to adjust serum or plasma levels of vitamin E for serum cholesterol (18). In addition to the variables in the first model, the second model included *a priori* selected potential confounders (sex, maternal smoking during pregnancy, maternal allergic history, number of older siblings) and those based on a bivariate association ( $P$ -value < 0.25) with any of the endpoints (maternal age, maternal education, maternal smoking during first year, day care attendance during first year, use of antibiotic during the first year, duration of breastfeeding, paternal allergic history, and paternal education).

Log to base 10 values of the alpha and gamma tocopherol were used. In sensitivity analyses, vitamin E was also categorized as quartiles, but the results were similar to those based on log to base 10. A test of interaction between vitamin E and follow-up time in relation to atopic sensitization and wheezing was evaluated. For heterogeneity test between the study centers, an interaction term between centers and vitamin E was evaluated for all endpoints. Statistical significance for the associations between vitamin E and the endpoints was set at  $P < 0.05$ . The analyses were performed using STATA 11 (StataCorp LP, College Station, TX, USA).

## Results

### Characteristics of the study population

Of the study participants ( $n = 1133$ ), over 80% of the children returned the questionnaires during the follow-up visits between ages of 1 and 6 years, although the proportion of children who underwent blood sampling for IgE measurements was only 63 and 66% at ages 4.5 and 6 years, respectively (Table 1). The prevalence of sensitization to food and

inhalant allergens increased from 8 and 21% at age 1 year to 30 and 33% at 6 years, respectively. The annual incidence of atopic dermatitis and prevalence of wheezing decreased during the follow-up, while the annual incidence of asthma somewhat fluctuated during the follow-up and was only 1% at 6 years (Table 1). Current asthma at 6 years ( $n = 61$ ) was 5.4%.

There were several significant differences in the risk of the endpoints between the study centers: The risk of atopic sensitization was higher in Finnish children compared with Austrian children; the risk of atopic dermatitis was higher in France and Finland, the risk of wheezing without cold higher in Switzerland, France, and Finland, and the risk of asthma higher in France and Germany when compared to Austrian children (Table 2). Girls were less likely to have wheezing and asthma than the boys did. Having one or more siblings increased the risk of wheezing without cold and asthma. The concentrations of alpha tocopherol were highest in Finland but lowest in German children; the concentrations of gamma tocopherol were highest in Austria, but lowest in France. The concentrations of alpha tocopherol were higher in girls than in boys (Table 2).

### Associations between serum vitamin E concentrations and the endpoints

Neither alpha nor gamma tocopherol concentrations were statistically significantly associated with atopic sensitization and atopic dermatitis (Table 3). In unadjusted models, alpha tocopherol was significantly inversely associated with wheezing symptoms and borderline inversely associated with wheezing without cold (Table 4). After adjustment for confounding variables, the associations remained only at borderline significance for both endpoints. Alpha tocopherol concentrations were not associated with asthma. Gamma tocopherol was not associated with wheezing or asthma (Table 4).

### Results of interaction models

We did not observe any significant interactions between follow-up time and either alpha or gamma tocopherol in relation to wheezing. There were, however, some interactions between time and vitamin E in relation to atopic sensitization, particularly between alpha tocopherol and atopic sensitization to any allergen ( $P = 0.061$ ) and any food allergen ( $P = 0.040$ ), suggesting a negative association at age 1 year and positive associations at 4.5 and 6 years. However, these associations were not statistically significant when the analyses were performed cross-sectionally at 1, 4.5, or 6 years (data not shown).

There was no statistically significant heterogeneity between the study centers and vitamin E concentrations in relation to the endpoints, except for a suggestive statistically significant interaction between alpha tocopherol and the study centers in relation to inhalant atopic sensitization ( $P = 0.17$ ) and between gamma tocopherol and the study centers in relation to atopic dermatitis ( $P = 0.062$ ), as well as any wheezing

**Table 1** Prevalence of atopic sensitization and wheezing symptoms, and the incidence of new atopic dermatitis and new asthma between two questionnaire surveys

| Outcomes                                        | Year 1<br>N = 939*/1045†<br>n (%) | Year 1.5<br>N = 1003†<br>n (%) | Year 2<br>N = 1014†<br>n (%) | Year 3<br>N = 982†<br>n (%) | Year 4/4.5<br>N = 717*/994†<br>n (%) | Year 5<br>N = 966†<br>n (%) | Year 6<br>N = 745*/934†<br>n (%) |
|-------------------------------------------------|-----------------------------------|--------------------------------|------------------------------|-----------------------------|--------------------------------------|-----------------------------|----------------------------------|
| Sensitization to any allergen                   | 264 (25)                          | –¶                             | –¶                           | –¶                          | 327 (46)                             | –¶                          | 340 (46)                         |
| Sensitization to any food allergen‡             | 87 (8.3)                          | –¶                             | –¶                           | –¶                          | 199 (28)                             | –¶                          | 221 (30)                         |
| Sensitization to any inhalant allergen§         | 217 (21)                          | –¶                             | –¶                           | –¶                          | 234 (33)                             | –¶                          | 243 (33)                         |
| New doctor-diagnosed atopic dermatitis**        | 103 (9.9)‡‡                       | 38 (3.9)                       | 25 (2.5)                     | 22 (2.2)                    | 21 (2.1)                             | 9 (1.0)                     | 13 (1.4)                         |
| Wheezing without cold during the past 12 months | 97 (9.3)‡‡                        | 39 (3.9)                       | 35 (3.5)                     | 42 (4.3)                    | 53 (5.3)                             | 38 (3.9)                    | 48 (5)                           |
| Any wheezing symptom during the past 12 months  | 289 (28)‡‡                        | 169 (17)                       | 154 (15)                     | 168 (17)                    | 150 (15)                             | 122 (13)                    | 102 (11)                         |
| New doctor-diagnosed asthma††                   | 46 (4.4)‡‡                        | 37 (3.7)                       | 16 (1.6)                     | 36 (3.7)                    | 26 (2.6)                             | 19 (2.0)                    | 9 (1.0)                          |

\*The number of subjects who underwent blood sampling for IgE measurements at each year (used as the denominator for calculating prevalence of allergic sensitization).

†The number of subjects who answered the questionnaire at each year (used as the denominator for calculating the prevalence of wheezing and incidence of asthma).

‡Sensitization (IgE  $\geq 0.35$  kU/l at year 1 and IgE  $\geq 0.70$  kU/l at year 4.5 and 6) to any of hen's egg, cow's milk, peanut, hazelnut, carrot, and wheat.

§Sensitization (IgE  $\geq 0.35$  kU/l at year 1 and IgE  $\geq 0.70$  kU/l at year 4.5 and 6) to any of house dust mites (*Dermatophagoides pteronyssinus* and *D. farinae*), alder, birch, European hazel, grass pollen mixture, rye, mugwort, plantain, cat, horse and dog dander, and the mold *Alternaria alternata*.

¶Not assessed during this year.

\*\*Incidence of parental-reported doctor-diagnosed atopic dermatitis at least once.

††Incidence of parental-reported doctor-diagnosed asthma at least once or/and doctor-diagnosed asthmatic (or obstructive) bronchitis more than once.

‡‡In the subsequent models, the atopic dermatitis, wheezing, and asthma endpoints at year 1 were not included in the analysis to estimate the prospective relationship between the background characteristics and vitamin E at year 1 and the endpoints.

symptom ( $P = 0.14$ ). Stratification by center did not yield consistent estimates, and in some cases, the models did not converge, possibly because of small number of endpoints at each center (data not shown).

## Discussion

In this study, we found no association between serum concentrations of vitamin E at the age of 1 year and longitudinal development of allergies and asthma up to age 6 years, except for border-line inverse associations between alpha tocopherol and wheezing symptoms. The results were generally consistent across the study centers.

The strengths of this study include its multicenter design, a unique and well-defined population unselected on the basis of allergic risk, its prospective nature and longitudinal assessments of the allergic and asthma endpoints. The measurement of vitamin E before the assessment of the endpoints and the exclusion of the endpoints occurring before and during year 1 (year of vitamin E measurement) ensured the estimation of the prospective relationship between the exposure and the atopic dermatitis, wheezing, and asthma endpoints. However, we only measured vitamin E at one time point, at age 1 year. A repeated assessment of vitamin E during early life may clarify its putative role in the development of the diseases.

All previous studies that have examined the association between serum vitamin E concentrations and the development of allergic diseases and asthma in children have been cross-sectional or retrospective (case-control) studies, and the results are conflicting (10–15). In a cross-sectional survey of US children aged 4–16 years, there was no association between vitamin E concentrations and the risk of asthma (15). Similarly, another cross-sectional US study of children 6–17 years also reported no association between serum vitamin E levels and the risk of asthma (14). In a Taiwanese case-control study, no association was observed between serum vitamin E concentrations and the risk of allergies in children (11), but other case-control studies have reported a decreased risk of atopic eczema with higher serum concentrations of alpha tocopherol (12, 13), but not for asthma (12). The case-control studies were of small sample sizes and conducted in hospital or school population, which limit the generalizability of findings. One cross-sectional US study (19) investigated the association between dietary antioxidant intake from fruits and vegetables and lung function performance in children aged 11–16 years. Low intake was associated with lower forced expiratory flow between 25% and 75% (19).

As allergies precede oxidative stress, it is impossible to establish whether the level of antioxidant concentrations preceded the onset of allergic diseases in cross-sectional and

**Table 2** Relation of the background characteristics to the allergic endpoints (odds ratio [OR] and 95% confidence interval [95% CI]) and serum concentrations of alpha and gamma tocopherols at 1 year

| Characteristic            | N* = 1133<br>n (%) | Sensitization<br>to any allergen<br>OR (95% CI)† | Doctor-diagnosed<br>atopic dermatitis<br>OR (95% CI)‡ | Wheezing<br>apart from cold<br>OR (95% CI)† | Any wheezing<br>symptom<br>OR (95% CI)† | New doctor-<br>diagnosed<br>asthma<br>OR (95% CI)‡ | Current asthma at<br>age 6 years<br>OR (95% CI)‡§ | Serum alpha<br>tocopherol |                 | Serum gamma<br>tocopherol |                 |
|---------------------------|--------------------|--------------------------------------------------|-------------------------------------------------------|---------------------------------------------|-----------------------------------------|----------------------------------------------------|---------------------------------------------------|---------------------------|-----------------|---------------------------|-----------------|
|                           |                    |                                                  |                                                       |                                             |                                         |                                                    |                                                   | Mean (SD) mg/l¶           | Mean (SD) mg/l¶ | Mean (SD) mg/l¶           | Mean (SD) mg/l¶ |
| Study center              |                    |                                                  |                                                       |                                             |                                         |                                                    |                                                   |                           |                 |                           |                 |
| Austria                   | 220 (19)           | 1                                                | 1                                                     | 1                                           | 1                                       | 1                                                  | 1                                                 | 7.2 (2.1)                 | 7.2 (2.1)       | 0.7 (0.4)                 | 0.7 (0.4)       |
| Switzerland               | 242 (21)           | 1.28 (0.93–1.76)                                 | 0.63 (0.39–1.03)                                      | 2.24 (1.28–3.92)                            | 1.19 (0.87–1.64)                        | 1.25 (0.73–2.12)                                   | 1.02 (0.58–1.79)                                  | 6.4 (2.4)                 | 6.4 (2.4)       | 0.5 (0.3)                 | 0.5 (0.3)       |
| France                    | 203 (18)           | 1.11 (0.81–1.54)                                 | 2.55 (1.71–3.79)                                      | 2.41 (1.37–4.26)                            | 1.32 (0.95–1.82)                        | 2.33 (1.41–3.84)                                   | 1.40 (0.80–2.45)                                  | 5.8 (2.3)                 | 5.8 (2.3)       | 0.4 (0.2)                 | 0.4 (0.2)       |
| Germany                   | 254 (22)           | 1.09 (0.80–1.50)                                 | 0.75 (0.47–1.19)                                      | 1.39 (0.76–2.52)                            | 1.22 (0.89–1.67)                        | 2.54 (1.59–4.06)                                   | 2.06 (1.26–3.36)                                  | 5.4 (1.8)                 | 5.4 (1.8)       | 0.5 (0.3)                 | 0.5 (0.3)       |
| Finland                   | 214 (19)           | 1.63 (1.19–2.34)                                 | 1.80 (1.19–2.73)                                      | 2.86 (1.64–4.96)                            | 1.08 (0.77–1.51)                        | 1.28 (0.74–2.22)                                   | 1.05 (0.58–1.88)                                  | 8.3 (2.0)                 | 8.3 (2.0)       | 0.5 (0.2)                 | 0.5 (0.2)       |
| P-value                   |                    | 0.019                                            | <0.001                                                | 0.001                                       | 0.492                                   | <0.001                                             | 0.007                                             | <0.001                    | <0.001          | <0.001                    | <0.001          |
| Sex                       |                    |                                                  |                                                       |                                             |                                         |                                                    |                                                   |                           |                 |                           |                 |
| Boy                       | 560 (49)           | 1                                                | 1                                                     | 1                                           | 1                                       | 1                                                  | 1                                                 | 6.4 (2.3)                 | 6.4 (2.3)       | 0.5 (0.3)                 | 0.5 (0.3)       |
| Girl                      | 530 (47)           | 0.90 (0.74–1.10)                                 | 1.21 (0.93–1.57)                                      | 0.67 (0.49–0.92)                            | 0.67 (0.55–0.82)                        | 0.56 (0.42–0.76)                                   | 0.58 (0.41–0.81)                                  | 7.1 (2.4)                 | 7.1 (2.4)       | 0.6 (0.3)                 | 0.6 (0.3)       |
| Missing information       | 43 (4)             |                                                  |                                                       |                                             |                                         |                                                    |                                                   |                           |                 |                           |                 |
| P-value                   |                    | 0.307                                            | 0.165                                                 | 0.012                                       | <0.001                                  | <0.001                                             | 0.001                                             | <0.001                    | <0.001          | 0.156                     | 0.156           |
| Maternal allergic history |                    |                                                  |                                                       |                                             |                                         |                                                    |                                                   |                           |                 |                           |                 |
| No                        | 694 (61)           | 1                                                | 1                                                     | 1                                           | 1                                       | 1                                                  | 1                                                 | 6.6 (2.2)                 | 6.6 (2.2)       | 0.5 (0.3)                 | 0.5 (0.3)       |
| Yes                       | 439 (39)           | 1.18 (0.97–1.44)                                 | 1.62 (1.24–2.10)                                      | 1.90 (1.40–2.58)                            | 1.56 (1.28–1.90)                        | 1.91 (1.43–2.55)                                   | 2.05 (1.47–2.84)                                  | 6.9 (2.6)                 | 6.9 (2.6)       | 0.5 (0.3)                 | 0.5 (0.3)       |
| P-value                   |                    | 0.094                                            | <0.001                                                | <0.001                                      | <0.001                                  | <0.001                                             | <0.001                                            | 0.099                     | 0.099           | 0.979                     | 0.979           |
| Siblings                  |                    |                                                  |                                                       |                                             |                                         |                                                    |                                                   |                           |                 |                           |                 |
| No sibling                | 413 (36)           | 1                                                | 1                                                     | 1                                           | 1                                       | 1                                                  | 1                                                 | 6.8 (2.3)                 | 6.8 (2.3)       | 0.6 (0.3)                 | 0.6 (0.3)       |
| One sibling               | 341 (30)           | 1.31 (1.03–1.67)                                 | 1.15 (0.83–1.59)                                      | 1.83 (1.22–2.73)                            | 1.33 (1.04–1.70)                        | 1.63 (1.14–2.33)                                   | 1.18 (0.80–1.76)                                  | 6.6 (2.3)                 | 6.6 (2.3)       | 0.5 (0.3)                 | 0.5 (0.3)       |
| 2 or more siblings        | 379 (34)           | 1.22 (0.96–1.54)                                 | 1.18 (0.86–1.62)                                      | 1.90 (1.29–2.82)                            | 1.26 (0.99–1.61)                        | 1.30 (0.91–1.87)                                   | 1.00 (0.67–1.49)                                  | 6.8 (2.5)                 | 6.8 (2.5)       | 0.5 (0.3)                 | 0.5 (0.3)       |
| P-value                   |                    | 0.072                                            | 0.553                                                 | 0.003                                       | 0.052                                   | 0.028                                              | 0.644                                             | 0.534                     | 0.534           | 0.165                     | 0.165           |

\*Total number of children who originally participated in the study.

†Analysis carried out using generalized estimating equations (GEE) to account for the repeated measures of the outcome with exchangeable correlation structure.

‡Analysis carried out using discrete-time hazard model.

§'Current asthma' defined as incident asthma during follow-up PLUS wheeze or use of asthma medication during the 12 months preceding the age of 6 years.

¶Analysis carried out using the Student's *t*-test for characteristics with two categories and ANOVA for characteristics with more than two categories.

**Table 3** Association between vitamin E concentrations at year 1 and the risk of allergic sensitization and atopic dermatitis by the age of 6 years: Odds ratio (OR) and 95% confidence interval (95% CI)

| Vitamin E ( $\log_{10}$ ) | Sensitization to any allergen |                  | Sensitization to any food allergen |                  | Sensitization to any inhalant |                  | Atopic dermatitis OR (95% CI)† |                   |
|---------------------------|-------------------------------|------------------|------------------------------------|------------------|-------------------------------|------------------|--------------------------------|-------------------|
|                           | Model I‡                      | Model II§        | Model I‡                           | Model II§        | Model I‡                      | Model II§        | Model I‡                       | Model II§         |
| $\alpha$ -tocopherol      | 1.29 (0.69–2.40)              | 1.32 (0.71–2.48) | 0.97 (0.48–1.96)                   | 1.08 (0.53–2.21) | 1.18 (0.61–2.98)              | 1.19 (0.61–2.32) | 3.35 (0.69–16.30)              | 3.28 (0.61–17.65) |
| P-value                   | 0.426                         | 0.383            | 0.925                              | 0.831            | 0.620                         | 0.609            | 0.134                          | 0.167             |
| $\gamma$ -tocopherol      | 0.80 (0.47–1.37)              | 0.82 (0.48–1.42) | 0.66 (0.35–1.24)                   | 0.67 (0.36–1.25) | 0.91 (0.52–1.62)              | 0.93 (0.52–1.65) | 1.29 (0.43–3.86)               | 1.25 (0.41–3.87)  |
| P-value                   | 0.411                         | 0.478            | 0.195                              | 0.212            | 0.756                         | 0.797            | 0.651                          | 0.695             |

\*Analyzed using generalized estimating equations to account for the repeated measures of the outcome with exchangeable correlation structure.

†Analyzed using discrete-time hazard model.

‡Adjusted for time (only for the allergic sensitization endpoints), cholesterol, farming status, and center of study.

§Adjusted model including time (only for the allergic sensitization endpoints), cholesterol, center of study, farming status, sex, maternal smoking during pregnancy, maternal allergic history, number of older siblings, maternal age, maternal education, maternal smoking during first year, day care attendance during first year, use of antibiotic during first year, duration of breastfeeding, paternal allergic history, and paternal education.

**Table 4** Association between vitamin E concentration at year 1 and the risk of wheezing, and asthma by the age of 6 years: Odds ratio (OR) and 95% confidence interval (95% CI)

| Vitamin E ( $\log_{10}$ ) | Wheezing without cold |                  | Any Wheezing symptom |                  | New doctor-diagnosed asthma |                  | Current asthma at age 6 years‡ |                  |
|---------------------------|-----------------------|------------------|----------------------|------------------|-----------------------------|------------------|--------------------------------|------------------|
|                           | Model I¶              | Model II**       | Model I¶             | Model II**       | Model I¶                    | Model II**       | Model I¶                       | Model II**       |
| $\alpha$ -tocopherol      | 0.43 (0.18–1.06)      | 0.45 (0.19–1.09) | 0.44 (0.24–0.90)     | 0.52 (0.27–1.02) | 0.88 (0.31–2.51)            | 0.92 (0.32–2.65) | 1.10 (0.36–3.31)               | 1.15 (0.38–3.48) |
| P-value                   | 0.067                 | 0.076            | 0.023                | 0.057            | 0.810                       | 0.873            | 0.869                          | 0.806            |
| $\gamma$ -tocopherol      | 0.92 (0.34–2.49)      | 0.75 (0.28–1.98) | 0.92 (0.48–1.76)     | 0.96 (0.50–1.85) | 1.64 (0.64–4.23)            | 1.68 (0.63–4.51) | 1.72 (0.67–4.40)               | 1.85 (0.70–4.90) |
| P-value                   | 0.869                 | 0.562            | 0.798                | 0.903            | 0.304                       | 0.300            | 0.256                          | 0.215            |

\*Analysis carried out using generalized estimating equations to account for the repeated measures of the outcome with exchangeable correlation structure.

†Analysis carried out using discrete-time hazard model.

‡Current asthma at 6 years defined as incident asthma during follow-up PLUS wheeze or use of asthma medication during 12 months prior to 6 years.

¶Model adjusted for time (only for the wheezing outcomes), cholesterol, farming status, and center of study.

\*\*Fully adjusted model including time (only for the wheezing outcomes), cholesterol, center of study, farming status, sex, maternal smoking during pregnancy, maternal allergic history, number of older siblings, maternal age, maternal education, maternal smoking during first year, day care attendance during first year, use of antibiotic during first year, duration of breastfeeding, paternal allergic history, and paternal education.

case-control studies as the above studies (6). Some studies have reported a decreased risk of allergies and asthma in children with maternal dietary vitamin E intake during pregnancy (20–23), although other studies have reported no association (24, 25). The food frequency questionnaire (FFQ) method, which has been used in these studies, is less sensitive in measuring the intake of vitamin E compared with many other nutrients and may poorly reflect serum levels of vitamin E (6).

Although we observed a marginal inverse association between alpha tocopherol and wheezing symptoms, there was lack of associations between vitamin E and asthma and atopy. These results are contrary to the findings from the previous studies (10–15). The cross-sectional and case-control designs of the previous studies may limit their comparison with the current study. In addition, the timing of vitamin E measurement and the differing ages of the children in the different studies may also explain the discrepancies in results. There may be a critical time window of exposure to antioxidants at which the risk of allergies is maximized, and this may explain the disparities among studies (3, 24). Although detecting such a critical time window may be challenging, future studies could be enhanced by including repeated measurement of vitamin E during early life. This will help to determine the possible time point of exposure, as well as the long-term exposure, to vitamin E in relation to the risk of the diseases (24).

Serum concentrations of vitamin E seem highly age-dependent; hence, its antioxidant effects on the immune system may also be age-dependent (5). Therefore, in examining the association between vitamin E and the risk of allergies and asthma in children, it is important to take into account the age of the subjects when generalizing to other age groups. The serum vitamin E concentrations in the present study and the previous studies (10–15) varied considerably, which may reflect the varied age of the subjects at time of vitamin E assessment in the different studies. The children from farming and nonfarming environments did not differ in the present study.

The role of vitamin E in the development of allergies and asthma may occur through other biological pathways, which may be independent of its antioxidative properties (3, 26). This is highlighted by the lack of putative associations between other antioxidants, such as vitamin C and carotenoids and the development of allergies and lung function performance (3). Although the immunological and inflammatory properties of vitamin E are unclear, the nonantioxidant biological pathways in inflammation may be mediated by inhibiting protein kinase B and C activities (3). This in turn may inhibit the activation

and binding of the DNA transcription factor NF- $\kappa$ B and consequently affect the regulation of inflammatory cytokines, adhesion, and other pro-inflammatory molecules (3). Because empirical evidence highlighting this proposal is lacking, further immunological studies are required to understand the mechanisms underpinning vitamin E actions on the immune system and its inflammatory properties.

## Conclusions

Serum vitamin E concentrations at year 1 did not influence the risk of atopy, wheezing, or asthma by 6 years of age. On the one hand, further prospective studies with repeated measurements of vitamin E are needed to decipher its putative role in the development of allergies and asthma in childhood. By this, possible critical time window of exposure and potential age-dependency in vitamin E concentrations in relation to the development of allergies and asthma can be evaluated. On the other hand, the present findings may as well indicate that the antioxidant hypothesis for the onset of childhood allergies and asthma does not hold.

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## Author contributions

Bright I. Nwaru performed the data analyses and drafted the first version of the article; Suvi M. Virtanen, Juha Pekkanen, and Anne M. Karvonen contributed to data collection, study management, data analysis, and drafting of the article; Gerge Alftan, Jon Genuneit, Roger Lauener, Jean-Charles Dalphin, Anne Hyvärinen, Petra Pfefferle, Josef Riedler, Julianne Weber, Caroline Roduit, Vincent Kaulek, and Charlotte Braun-Fahrlander contributed to data collection and study management, review of the article, and revising it critically for important intellectual content; Erika von Mutius contributed to data collection and was responsible for the design of the PASTURE study and review of the article and revising it critically for important intellectual content.

## Conflicts of interest

The authors declared no conflicts of interest.

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## Appendix

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