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Serum carotenoid and tocopherol concentrations and risk of asthma in childhood: a nested case–control study

N. Hämäläinen¹, B. I. Nwaru^{1,2} , I. Erlund³, H.-M. Takkinen^{1,4}, S. Ahonen^{1,5,6}, J. Toppari^{7,8}, J. Ilonen^{9,10}, R. Veijola¹¹, M. Knip^{12,13,14}, M. Kaila^{15,16} and S. M. Virtanen^{1,4,5,6}

¹School of Health Sciences, University of Tampere, Tampere, Finland, ²Asthma UK Centre for Applied Research, Centre for Medical Informatics, Usher Institute of Population Health Sciences and Informatics, The University of Edinburgh, Edinburgh, UK, ³The Genomics and Biomarkers Unit, Department of Health, National Institute for Health and Welfare, Helsinki, Finland, ⁴Nutrition Unit, Department of Health, National Institute for Health and Welfare, Helsinki, Finland, ⁵Tampere Center for Child Health Research, School of Medicine, Tampere University Hospital, University of Tampere, Tampere, Finland, ⁶The Science Centre of Pirkanmaa Hospital District, Tampere, Finland, ⁷Department of Physiology, Institute of Biomedicine, University of Turku, Turku, Finland, ⁸Department of Pediatrics, Turku University Hospital, Turku, Finland, ⁹Immunogenetics Laboratory, Faculty of Medicine, University of Turku and Turku University Hospital, Turku, Finland, ¹⁰Department of Clinical Microbiology, University of Eastern Finland, Kuopio, Finland, ¹¹Department of Pediatrics, PEDEGO Research Unit, Medical Research Center, Oulu University Hospital and University of Oulu, Oulu, Finland, ¹²Children's Hospital, University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland, ¹³Folkhälsan Research Center, Helsinki, Finland, ¹⁴Research Programs Unit, Diabetes and Obesity, University of Helsinki, Helsinki, Finland, ¹⁵Public Health Medicine, University of Helsinki and Helsinki University Hospital, University of Helsinki, Helsinki, Finland and ¹⁶Department of Pediatrics, Tampere University Hospital, Tampere, Finland

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Summary

Background The antioxidant hypothesis regarding the risk of asthma in childhood has resulted in inconsistent findings. Some data indicate that the role of antioxidants in childhood asthma risk may have a critical time window of effect, but only a well-designed longitudinal cohort study can clarify this hypothesis.

Objective To study the longitudinal associations between serum carotenoid and tocopherol concentrations during the first 4 years of life and asthma risk by the age of 5 years.

Methods Based on a case–control design nested within a Finnish birth cohort, 146 asthma cases were matched to 270 controls on birth time, sex, genetic risk, and birth place. Non-fasting blood samples were collected at the ages of 1, 1.5, 2, 3, and 4 years and serum carotenoids and tocopherols were analysed. Parents reported the presence and age at start of persistent doctor-diagnosed asthma in the child at the age of 5 years. Data analyses were conducted using generalized estimating equations.

Results We did not find strong associations between serum carotenoids and tocopherols and the risk of asthma based on age-specific and longitudinal analyses. Both lower and higher quarters of α -carotene and γ -tocopherol increased the risk of asthma.

Conclusions The current findings do not support the suggestion that the increased prevalence of asthma may be a consequence of decreased intake of antioxidant nutrients. Moreover, we did not confirm any critical time window of impact of antioxidants on asthma risk. Replication of these findings in similar longitudinal settings will strengthen this evidence base.

Keywords antioxidants, asthma, carotenoids, children, longitudinal study, tocopherols, vitamin A, vitamin E

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Correspondence:

Bright I. Nwaru, School of Health Sciences, University of Tampere, Tampere 33014, Finland.

E-mail: bright.nwaru@uta.fi

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Introduction

According to the dietary antioxidant hypothesis, the rise in asthma during the second half of the last century may partly be a consequence of decreased consumption

of foods rich in antioxidant nutrients [1–4]. Compared to non-asthma patients, oxidative stress in the circulation and airways of asthma patients is greater; this is believed to be a consequence of the activities of oxygen free radicals [5–9]. Nutritional antioxidants are required

by the lung, in addition to its intra- and extracellular antioxidant defence systems, to inhibit the propagation of the damages caused by oxygen free radicals [9]. Vitamins A and E are key antioxidant nutrients; carotenoids are the precursors of vitamin A, whilst α -tocopherol is the active form of vitamin E that is transported and used by the liver; these are thought to beneficially influence the risk of asthma and allergy.

There is evidence that maternal intake of vitamins A and E during pregnancy may decrease the risk of asthma and allergy in the offspring, although contrary evidence also exists [10–16]. Whilst maternal dietary transfer during pregnancy to the fetus takes place via the placenta, dietary transfers during infancy and childhood are through the breastmilk and the child's gut, respectively. Given these different routes of dietary transfer, the immunological effects of fetal dietary exposures may differ from dietary exposures during early life [17]. The associations between vitamin A and E exposures during early life and the risk of childhood asthma have been evaluated only by a few studies and results are inconsistent [18–24]. Most existing studies are however either retrospective case-control [18–20] or cross-sectional [21–24] in design. More prospective studies are required to elucidate the putative role of vitamins A and E during early life on the risk of childhood asthma.

Based on the PASTURE (Protection against allergy: study in rural environments) study, a multi-centre birth cohort study across rural farming settings in five European countries, we recently showed that serum α - and γ -tocopherol concentrations at the age of 1 year were not strongly associated with the risk of asthma and allergic disorders by the age of 6 years [25]. There are some suggestions that serum antioxidant levels may be age dependent and that its association with allergic disorders may have a critical time window of maximal effect [16, 25, 26]. In the PASTURE study and previous studies, serum antioxidants have been measured once; thus, it was not possible to evaluate potential critical time window or age dependency of effect. To implement such an analysis, a well-designed longitudinal cohort with repeated measurements of antioxidant nutrients would provide an ideal setting.

The Finnish Type 1 Diabetes Prediction and Prevention (DIPP) Nutrition study longitudinally investigates the role of fetal and early life diet in the development of type 1 diabetes, asthma and allergy, and overweight in childhood [27, 28]. In addition to dietary assessments during pregnancy and lactation, the child's dietary intake and serum nutrient levels have been repeatedly measured during early childhood. The longitudinal design of the DIPP birth cohort offers a unique opportunity to address the questions of possible age dependency and assess potential critical time window in the associations between antioxidants and the risk of

asthma in children. The aim of this study was to investigate the associations between serum carotenoid and tocopherol concentrations during the first 4 years of life and the risk of asthma by the age of 5 years.

Methods

Study population

The DIPP study is a multidisciplinary population-based prospective birth cohort that started in 1994 [27]. New-born infants with human leucocyte antigen (HLA)-conferred susceptibility to type 1 diabetes are recruited from Turku, Oulu, and Tampere University Hospitals and monitored at 3- to 12-month intervals for diabetes-associated antibodies, growth, and environmental exposures. In September 1996 and October 1997 in Oulu and Tampere, respectively, a nutrition study (DIPP Nutrition Study) was initiated within the DIPP study and that study examines the role of diet during pregnancy, lactation, infancy and early childhood in the development of type 1 diabetes, allergy, and asthma in childhood [28]. Children who were still participating in the dietary follow-up at the age of 5 years were invited to take part in the allergy component of the study involving completion of a modified version of the International Study of Asthma and Allergy in Children (ISAAC) questionnaire. The children also underwent blood sample analysis for the measurement of specific immunoglobulin E (IgE) responses for food and inhalant allergens. Of the 4075 children invited to the study, 3781 (93%) took part. The study was approved by the local ethics committees of the University Hospital of Tampere and the Ethics Committee of the Faculty of Medicine, University of Oulu, Finland (97193M), and the parents signed a written informed consent.

The current study is a case-control analysis nested within the DIPP Nutrition Study birth cohort. Each asthma case ($n = 146$) was matched to two randomly selected control children on birth time (within 3 months), sex, genetic risk for type 1 diabetes (high, moderate), and birth place (Tampere, Oulu). For the current study (which relates serum carotenoid and tocopherol levels between 1 and 4 years to the risk of asthma by 5 years), control children, as well as their corresponding cases, must have had nutrient serum biomarkers available at least from one time point before the case became a case. The control children must also have completed the ISAAC questionnaire and must not have asthma at the time the case became a case.

Assessment of serum carotenoids and tocopherols

Non-fasting blood samples were collected from the case and control children by venipuncture at the ages of 1,

1.5, 2, 3, and 4 years. The blood samples were protected from light during processing and stored at room temperature and allowed to clot for 30–60 min. Serum biomarkers of carotenoids and tocopherols were determined at the Biomarker Laboratory, National Institute for Health and Welfare with a micromethod on 50 µL aliquots with the reverse-phase high-pressure liquid chromatography by fluorescence at 292/324 nm. The precision between series was 5.0 coefficient variation (CV) % for alpha-tocopherol and 6.7 CV% for gamma-tocopherol. The serum samples were stored at -70°C . Serum cholesterol concentration was analysed manually by an Olli-C photometer using an enzymatic (CHOD-PAP) method. The laboratory personnel was unaware of the case-control status of the samples [29].

Outcome assessment

Parents completed a modified version of the ISAAC questionnaire on the child's history of allergic symptoms and asthma when the children were 5 years of age. Asthma cases were identified from the whole cohort, and a case was defined as having a doctor-diagnosed asthma plus either any wheezing symptom during the preceding 12 months or use of asthma medication during the preceding 12 months, thus having persistent asthma. Age at asthma diagnosis was determined by the question: 'at what age was asthma diagnosed?' We have previously validated this definition of asthma against the Social Insurance Institute reimbursement database on anti-asthmatic medications, with excellent validity estimates [30].

Statistical analysis

Descriptive statistics were computed using simple frequencies, chi-square tests, means, and standard deviations. The repeated measurement of serum carotenoids and tocopherols imposed a unique design feature for the current study, so that classical conditional logistic regression was not suitable to study the longitudinal associations between carotenoid and tocopherol serum levels and the risk of asthma, as conditional logistic regression utilizes one measurement per each individual. The repeated serum measurements imply that nutrient levels will correlate within the same individual. To account for this, we employed the generalized estimating equation (GEE) framework accompanied by the sandwich estimator of variance in order to estimate the regression coefficients. The GEE yields consistent regression coefficients, valid standard errors, and improved precision of the estimates [31]. In addition to the longitudinal analysis, we also analysed the association between serum carotenoid and tocopherol at the ages of 1, 2,

and 3 years and the risk of asthma using logistic regression. Very few asthma cases occurred after year 3; hence, we did not undertake the yearly analyses after the age of 3 years. We categorized serum carotenoid and tocopherol concentrations in quarters and combined the two mid-quarters, which was then used as the reference category.

In multivariable models, we adjusted for the following potential confounding variables selected on the basis of previous studies and on statistical tests: maternal age, maternal vocational education, number of siblings, duration of gestation, mode of delivery, birthweight, maternal smoking during pregnancy, parental allergy, atopic eczema by 6 months, duration of total breastfeeding, and season of birth. The associations with the tocopherols were additionally adjusted for serum cholesterol. To explore potential age-dependent effect and possible critical time window of effect, we performed interaction tests between serum nutrient levels and time in relation to the risk of asthma. We also tested the interaction between serum nutrient levels and total duration of breastfeeding in relation to the risk of asthma, as well as between serum nutrient levels and atopic eczema by 6 months of age. Statistical significance of the interaction tests was fixed at $P < 0.20$. Statistical significance of other analyses was taken as $P < 0.05$. SAS software (version 9.2, SAS Institute) was used in the analyses.

Results

Descriptive results

The study population included 416 children (146 asthma case and 270 matched-control children). The case and control children were similar with regard to the distribution of most demographic characteristics studied except for parental allergy and having atopic eczema by the age of 6 months: asthma cases were more likely to have parents with allergy and more likely to have atopic eczema by 6 months of age than the controls (Table 1). The mean serum α - and γ -carotene levels appeared to decrease over time between the ages of 1 and 4 years, whilst alpha- and gamma-tocopherol levels appeared to increase during the same time (Table 2).

Associations between carotenoids and asthma

In the analyses at each age group, lower and upper quarters of α -carotene at the ages of 3 and 4 years were associated with an increased risk of asthma (Table 3). However, at the age of 4 years, there were very few cases, and therefore, we could not draw confident

Table 1. Distribution of background characteristics by asthma case and control children

Characteristic	Frequency <i>N</i> = 416 <i>n</i> (%)	Cases <i>n</i> = 146 <i>n</i> (%)	Controls* <i>n</i> = 270 <i>n</i> (%)	<i>P</i> -value
Maternal vocational education				
None	15 (3.6)	4 (3)	11 (4)	0.671
Vocational education or course	113 (27)	41 (29)	72 (27)	
Secondary vocational education	187 (45)	69 (48)	118 (44)	
University studies or degree	94 (23)	29 (20)	65 (24)	
Missing information	7 (1.7)			
Parental allergy				
No	66 (16)	14 (10)	52 (20)	0.008
Yes	329 (79)	126 (90)	203 (62)	
Missing information	21 (5)			
Maternal smoking during pregnancy				
No	366 (88)	131 (92)	235 (91)	0.769
Yes	36 (9)	12 (8)	24 (9)	
Missing information	14 (3)			
Duration of gestation (weeks)				
Preterm	28 (7)	15 (10)	13 (5)	0.107
Term	370 (89)	126 (87)	244 (92)	
Post-term	13 (3)	4 (3)	9 (3)	
Missing information	5 (1)			
Number of siblings				
None	174 (42)	64 (45)	110 (42)	0.147
One	144 (35)	53 (37)	91 (34)	
Two	65 (16)	22 (16)	43 (16)	
Three or more	23 (6)	3 (2)	20 (8)	
Missing information	10 (2)			
Season of birth				
Spring (April – May)	80 (19)	28 (19)	52 (19)	0.710
Summer (June – August)	95 (23)	29 (20)	66 (25)	
Autumn (September–November)	85 (20)	30 (21)	55 (20)	
Winter (December–March)	156 (38)	59 (40)	97 (36)	
Mode of delivery				
Vaginal	351 (84)	123 (85)	228 (86)	0.808
Caesarean section	60 (14)	22 (15)	38 (14)	
Missing information	5 (1)			
Birthweight, grams (g)				
First quarter (< 3280 g)	101 (24)	46 (32)	55 (21)	0.074
Second quarter (3280–3599 g)	99 (24)	35 (24)	64 (24)	
Third quarter (3600–3890 g)	109 (26)	33 (23)	76 (29)	
Fourth quarter (> 3890 g)	102 (25)	31 (21)	71 (27)	
Missing information	5 (1)			
Atopic eczema by 6 months of age				
No	340 (82)	105 (73)	235 (88)	< 0.001
Yes	70 (17)	39 (27)	31 (11)	
Missing information	6 (1)			
Duration of total breastfeeding				
< 6 months	162 (39)	60 (42)	102 (38)	0.475
≥ 6 months	250 (60)	84 (58)	166 (62)	
Missing information	4 (1)			

*16 children served as controls for more than one case but were included only once in the total number of controls. Five of the controls became a case at a later date and were counted only as cases.

conclusions based on the results. When the associations were studied longitudinally, taking into account serum nutrient levels between the ages of 1 and 4 years and

adjusted for potential confounding factors, there were no statistically significant associations between serum carotenoid concentrations and asthma risk (Table 4).

Table 2. Mean (standard deviation, SD) serum carotenoid and tocopherol concentrations between 1 and 4 years of case and control children

	1 year Mean (SD)		1.5 years Mean (SD)		2 years Mean (SD)		3 years Mean (SD)		4 years Mean (SD)	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
α -carotene	0.35 (0.30)	0.34 (0.26)	0.21 (0.16)	0.22 (0.16)	0.14 (0.11)	0.16 (0.18)	0.12 (0.09)	0.13 (0.15)	0.11 (0.09)	0.12 (0.08)
β -carotene	1.05 (0.84)	1.07 (0.80)	0.72 (0.49)	0.73 (0.47)	0.53 (0.38)	0.59 (0.53)	0.53 (0.32)	0.53 (0.37)	0.49 (0.23)	0.52 (0.59)
α -tocopherol	7.99 (1.63)	7.26 (1.55)	6.74 (0.56)	6.75 (1.32)	7.09 (1.45)	6.78 (1.16)	7.12 (1.52)	7.01 (1.36)	7.13 (1.46)	7.46 (1.23)
γ -tocopherol	0.53 (0.25)	0.64 (0.32)	0.56 (0.26)	0.59 (0.32)	0.66 (0.33)	0.66 (0.28)	0.73 (0.39)	0.80 (0.33)	0.75 (0.39)	0.75 (0.27)

Associations between tocopherols and asthma

In the analyses at each age group, there were no associations with serum tocopherol concentrations and asthma risk except an increased risk of asthma with lower concentration of α -tocopherol at the age of 4 years (Table 3). However, at the age of 4 years, there were very few cases, and therefore, we could not draw reliable conclusions based on the results. In the longitudinal analyses and after adjustment for potential

confounding factors, only lower serum concentrations of γ -tocopherol were associated with an increased risk of asthma (Table 4).

Discussion

Summary of main findings

In this birth cohort with repeated assessment of vitamins A and E between 1 and 4 years of age, overall we

Table 3. Associations between serum carotenoid and tocopherol concentrations at 1, 2, and 3 years and the risk of asthma at 5 years[‡]

	1 year of age		2 years of age		3 years of age	
	Unadjusted* OR (95% CI) (n = 132 cases, 269 controls)	Adjusted* [†] OR (95% CI) (n = 120 cases, 234 controls)	Unadjusted* OR (95% CI) (n = 111 cases, 225 controls)	Adjusted* [†] OR (95% CI) (n = 102 cases, 203 controls)	Unadjusted* OR (95% CI) (n = 78 cases, 157 controls)	Adjusted* [†] OR (95% CI) (n = 73 cases, 138 controls)
α -carotene						
First quarter	1.18 (0.71–1.95)	0.97 (0.50–1.90)	1.01 (0.56–1.81)	0.90 (0.41–1.99)	2.90 (1.35–6.19)	4.43 (1.28–15.32)
Second and third quarters	1	1	1	1	1	1
Fourth quarter	1.37 (0.82–2.27)	1.41 (0.72–2.74)	0.96 (0.50–1.84)	0.78 (0.33–1.87)	2.07 (1.03–4.20)	2.40 (0.80–7.22)
P-value	0.476	0.542	0.989	0.860	0.015	0.057
β -carotene						
First quarter	1.42 (0.85–2.38)	1.02 (0.54–1.95)	1.22 (0.71–2.10)	1.28 (0.59–2.78)	1.35 (0.67–2.74)	1.37 (0.47–4.00)
Second and third quarters	1	1	1	1	1	1
Fourth quarter	1.43 (0.86–2.39)	1.36 (0.68–2.71)	0.96 (0.55–1.67)	1.00 (0.43–2.32)	1.52 (0.73–3.15)	1.51 (0.51–4.50)
P-value	0.256	0.672	0.718	0.792	0.503	0.734
α -tocopherol						
First quarter	0.87 (0.51–1.47)	1.32 (0.67–2.59)	0.57 (0.31–1.05)	0.70 (0.32–1.56)	0.92 (0.45–1.90)	0.46 (0.15–1.43)
Second and third quarters	1	1	1	1	1	1
Fourth quarter	1.02 (0.58–1.77)	0.84 (0.39–1.78)	1.04 (0.58–1.84)	0.78 (0.37–1.67)	1.30 (0.60–2.84)	1.17 (0.40–3.39)
P-value	0.560	0.574	0.320	0.578	0.915	0.393
γ -tocopherol						
First quarter	0.91 (0.53–1.55)	1.17 (0.59–2.33)	1.54 (0.90–2.63)	2.10 (0.99–4.49)	1.76 (0.91–3.41)	2.35 (0.90–6.11)
Second and third quarters	1	1	1	1	1	1
Fourth quarter	1.06 (0.65–1.73)	1.25 (0.67–2.33)	1.37 (0.76–2.47)	2.02 (0.86–4.72)	1.28 (0.63–2.60)	1.97 (0.66–5.83)
P-value	0.579	0.760	0.382	0.085	0.419	0.173

*Serum α - and γ -tocopherols concentrations adjusted for serum cholesterol.

[†]Adjusted for maternal age, maternal vocational education, number of siblings, duration of gestation, mode of delivery, birthweight, maternal smoking during pregnancy, parental allergy, atopic eczema by 6 months, duration of total breastfeeding, and season of birth.

[‡]Very few asthma cases occurred after the age of 3 years, hence low power to undertake cross-sectional analyses at the age of 4 years.

Table 4. Associations between longitudinal serum carotene and tocopherol concentrations between 1 and 4 years of age and the risk of asthma by the age of 5 years

	Asthma by 5 years of age Odds ratio (95% confidence interval)	
	Unadjusted*	Adjusted†
α-carotene		
First quarter	1.40 (0.99–1.97)	1.48 (0.99–2.25)
Second and third quarters	1	1
Fourth quarter	1.16 (0.82–1.64)	1.29 (0.82–2.03)
<i>P</i> -value	0.127	0.099
β-carotene		
First quarter	1.43 (1.00–2.05)	1.30 (0.85–1.98)
Second and third quarters	1	1
Fourth quarter	1.09 (0.76–1.56)	1.20 (0.78–1.86)
<i>P</i> -value	0.140	0.365
α-tocopherol		
First quarter	0.70 (0.48–1.02)	0.68 (0.43–1.09)
Second and third quarters	1	1
Fourth quarter	1.13 (0.80–1.60)	0.90 (0.60–1.35)
<i>P</i> -value	0.142	0.223
γ-tocopherol		
First quarter	1.16 (0.83–1.64)	1.50 (1.06–2.12)
Second and third quarters	1	1
Fourth quarter	0.97 (0.68–1.38)	1.23 (0.82–1.86)
<i>P</i> -value	0.677	0.031

*Alpha- and gamma-tocopherols adjusted for cholesterol.

†Adjusted for maternal age, maternal vocational education, number of siblings, duration of gestation, mode of delivery, birthweight, maternal smoking during pregnancy, parental allergy, atopic eczema by 6 months, duration of total breastfeeding, and season of birth.

observed no putative associations between serum carotenoids or tocopherols and the risk of asthma by the age of 5 years. Lower serum concentration of γ -tocopherol as well as lower and higher concentrations of α -carotene showed evidence of increased risk of asthma in children by the age of 5 years. Both lower and higher concentrations of α - and β -carotene were associated with an increased risk of asthma as well as lower and higher serum concentrations of α -tocopherol associated with a decreased risk, but the 95% confidence intervals for these associations were wide. These results were similar when the serum nutrient concentrations were analysed longitudinally and at each age of assessment, indicating no evidence of a critical time window for the effect of antioxidants on the risk of asthma.

Strengths and limitations of the study

The children included in the study were recruited from the two university hospitals where large proportion of births occur in Finland, an indication that the findings can be potentially generalized to the larger Finnish population of children of this age. The repeated

measurements of serum concentrations allowed the assessment of possible age-dependent effect of carotenoids and tocopherols on the risk of childhood asthma, a question of current concern in understanding whether early exposure to dietary nutrients has a critical time window of maximal impact on the risk of asthma and allergy later in childhood. The collection of serum data on carotenoids and tocopherols before the assessment of asthma minimized the possibility of bias with respect to subsequent reporting of asthma outcomes in the child. Asthma was measured using the standardized ISAAC questionnaire and has been validated within the DIPP cohort, showing that it reliably captured asthma when compared to clinical diagnosis [30].

Because the study children were recruited on the basis of HLA-conferred susceptibility to type 1 diabetes, this may limit the generalizability of our findings to children without HLA-conferred type 1 diabetes risk. Whilst in type 1 diabetes Th1 and Th17 lymphocytes play prominent roles, allergy and asthma are mediated through Th2 cell pathways, an indication that the increased risk for type 1 diabetes of our cohort may limit the application of our findings to the general population. However, the associations between HLA markers and the risk of allergy and asthma remained mixed [32, 33]. In the DIPP cohort, the absolute risk of type 1 diabetes is 3–4% up to the age of 15 years, whereas that in the Finnish population is close to 1% [27]. The recruitment of the study cohort was not based on a positive family history for allergy, which minimizes potential selection bias towards high-risk children for allergy. Moreover, the cumulative incidence of asthma in the DIPP cohort is comparative to the general Finnish population of children of this age [34, 35]. In some categories, we lacked overall statistical power to estimate reliable analyses; on the other hand, we could not analyse the exposure as a continuous variable as it is not linearly related to the outcome.

Comparison of study findings to previous studies

To our knowledge, this is the first study to undertake a longitudinal analysis of the role of vitamins A and E in the risk of asthma in childhood. Overall, we found no evidence to support the suggestion that early life exposure to antioxidant nutrients may reduce the risk of asthma, either longitudinally or at specific time points. Moreover, we did not find any evidence supportive of the suggestion that there may be a critical time window for the effect of antioxidant nutrients on the risk of asthma or any indication of age dependency of the potential effect. In the PASTURE birth cohort, we found no putative association between serum vitamin E concentrations at 1 year and risk of asthma and allergy by 6 years of age [25]. In other previous studies, the

findings have generally been mixed as both increased risk and null associations have been reported for vitamins A and E, but overall these findings have been primarily based on cross-sectional [21–23] and case-control [18–20, 36–39] studies, limiting their information value for the actual role of antioxidant nutrients in the asthma and allergy risk during childhood. In a recent systematic review, low vitamin A was associated with an increased risk of allergy, whilst a null finding was observed for vitamin E, but that systematic review did not differentiate the findings in children from that among adults [40]. Another systematic review suggested that low concentration of antioxidant nutrients may increase the risk of asthma and allergy in children, but recognized that almost all studies included were cross-sectional or case-control studies in design [41]. Two randomized controlled trials (RCT) that investigated the effect of vitamin A supplementation in early life on the risk of asthma and atopy later in childhood reported no significant effect [42, 43].

Potential mechanisms and implications of the findings

The antioxidant hypothesis that a decreased intake of antioxidant nutrients might have contributed to the observed increase in asthma and allergy during the latter half of the last century has been persuasive, considering the biological plausibility of the role of antioxidants in suppressing the respiratory damages resulting from oxidative stress emanating from oxygen free radicals [1–9]. However, the available evidence linking early life antioxidant nutrients to later asthma and allergy appears not supportive of this line of reasoning. Whereas the current study remains one of the well designed to evaluate both longitudinal and age-specific role of vitamins A and E in childhood asthma risk, the findings appear to be conclusively null. As highlighted in our last paper based on the findings from the PASTURE study [25], considering the overall trend of the evidence from different studies now undertaken, it is possible that the proposed antioxidant hypothesis does not hold, given that both observational and experimental studies have so far failed to definitely confirm the hypothesis. On the other hand, however, the evidence linking maternal antioxidant intake during pregnancy to the risk of asthma and allergy in the offspring appears promising, generally with a beneficial impact [44], consequently leading to initiation of an ongoing RCT to evaluate the effect of prenatal vitamin E for primary prevention of asthma and allergy in the offspring [45]. Whilst the results of that study will provide stronger data for the evidence base, it will also be important in highlighting whether fetal antioxidant exposures are more critical than exposures after birth for the risk of asthma and allergy later in childhood.

Conclusions

In this cohort of children with HLA-conferred susceptibility to type 1 diabetes, we found no strong associations between serum carotenoid and tocopherol concentrations during the first 4 years of life and asthma risk by 5 years, either longitudinally or when evaluated at specific ages. Moreover, we were not able to confirm the suggestion that the impact of antioxidants on the risk of asthma and allergy in childhood may have a critical time window of maximal effect. Whilst we advocate for more similar longitudinally designed studies to replicate the current results, the existing evidence however questions the validity of the antioxidant hypothesis regarding the risk of childhood asthma.

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Authors' contributions

NH, BIN, H-MT, and SMV designed, analysed, and prepared the manuscript. SMV designed and is responsible

for the DIPP Nutrition study and together with MKa for the DIPP Allergy study. SA and IE are responsible for data acquisition and preparation. RV, JI, MKn, and JT are responsible for data collection in the clinics and for paediatric expertise in this study. NH, BIN, IE, H-MT, SA, JT, JI, RV, MKn, MKa, and SMV participated in data analysis, interpretation, preparing and review of

the manuscript, and revising it critically for important intellectual content.

Conflict of interest

The authors declare no conflict of interest.

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