

1 Association between maternal carotenoids, vitamin A and vitamin E levels and allergic
2 outcomes in the offspring in the first 5 years of life

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36

37 **To the Editor,**

38 The development of allergic disease starts prenatally, a vital period during which maternal
39 exposure such as nutrient intake may modulate fetal and infant immune programming [1].
40 However, the role of various nutrients intake during maternal pregnancy, for instance, carotenoids,
41 vitamin A and vitamin E, in the development of allergic diseases in the offspring is inconclusive.
42 In this study, we aim to investigate the relationship between these nutrient levels in maternal
43 plasma at delivery and subsequent offspring allergic outcomes in the first 5 years of life in the
44 prospective mother-offspring cohort study Growing Up in Singapore Towards healthy Outcomes
45 (GUSTO).

46 The methodology of the GUSTO study has been described previously [2]. Briefly, we recruited
47 healthy pregnant mothers from two major public maternity units, the National University Hospital
48 and the KK Women's and Children's Hospital who agreed to enrol their offspring for future
49 follow-up. Ethics approval was obtained from the Domain Specific Review Board of Singapore
50 National Healthcare Group and the Centralised Institutional Review Board of SingHealth. Conduct
51 of this study was based on the guidelines in the Declaration of Helsinki. Informed consent was
52 obtained from the participants.

53 Maternal blood was obtained at delivery in a non-fasting state and stored in EDTA tubes at -80°C.
54 Ultra Performance Liquid Chromatography (UPLC) with Photo-Diode Array detection method
55 was used to determine plasma concentrations of Vitamin A (all-trans retinol), individual
56 carotenoids (α -carotene, β -carotene, β -cryptoxanthin, lutein, lycopene, and zeaxanthin), and
57 Vitamin E (α -, γ -, δ -tocopherols, and α -, γ -, δ -tocotrienols). The sample preparation, reagents and
58 chemicals used were as per previous HPLC method (1).

59 Parental reports of physician-diagnosed eczema, wheezing and rhinitis were captured by relevant
60 questionnaires, which have been described previously [3]. Questionnaires were administered at 3
61 weeks and 3, 6, 9, 12, 15, 18, 24, 36, 48, and 60 months. Family history of allergy was defined as
62 positive if the mother, father or an older sibling ever had eczema, asthma or allergic rhinitis.

63 Allergen sensitization was determined by skin prick testing (SPT). SPT to inhalant allergens
64 (house dust mites *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, and *Blomia*
65 *tropicalis*) and food allergens (egg, peanut and cow's milk) was carried out at 18, 36 and 60
66 months. At 60 months, SPT was also carried out to shrimp and crab allergens. All tests were

67 interpreted as positive if the wheal was at least 3 mm, and a child was considered as SPT-positive
68 if any one or more of the individual tests was positive with a positive reaction to the positive
69 control (histamine) and a negative reaction to the negative control (saline).

70

71 Statistical analysis was carried out using IBM SPSS version 25.0 (IBM, SPSS statistics, Armonk,
72 NK). Comparison of categorical demographic variables between the subjects included and
73 excluded from analysis was performed using Pearson's chi-square tests. The strength of
74 associations between maternal carotenoids status and allergic outcomes were assessed using
75 multivariable logistic regression using a p value of < 0.05 as significant; we report adjusted odds
76 ratios with exact 95% confidence intervals. Correction for multiple comparisons was carried out.

77 There were 701 subjects with maternal carotenoids, vitamin E and vitamin A levels analysed.
78 Majority of the study population were delivered by vaginal delivery (63.3%) with maternal
79 education levels of at least 12 years (61.2%, supplementary table 1). There were no differences in
80 gender, ethnicity, mode of delivery and family history of allergy between those included in the
81 analysis and those excluded. The subjects included in the study had higher proportion of mothers
82 with education levels of at least 12 years (61.2%) compared to those excluded (55.1%, $p < 0.05$,
83 supplementary table 1).

84 Increasing γ -tocotrienol and α -tocotrienol quartiles were associated with reduced risks of allergen
85 sensitization at 18 months (p-trend 0.05 and 0.03 respectively, Table 1). Comparing extreme
86 quartiles, infants in the highest quartile of γ -tocotrienol and α -tocotrienol had reduced risk of a
87 positive SPT at 18 months [adjusted odds ratio (95% CI) 0.3 (0.1-0.9) and 0.3 (0.1-0.8)
88 respectively] after adjustment for covariates (gender, ethnicity, maternal age, maternal BMI during
89 pregnancy, maternal education levels, multivitamin use during pregnancy and family history of
90 allergy). However, there were no significant associations after correction for multiple comparisons.

91 There was a trend of decreasing risk of eczema development in the first 5 years of life with
92 increasing quartiles of all trans retinol and γ -tocopherol albeit without statistical significance.
93 Comparing extreme quartiles, infants in the highest quartile of δ -tocopherol had reduced risk of
94 developing eczema by 18 months [adjusted odds ratio 0.4 (0.2-0.98)], after adjustment for
95 covariates. However, there were no significant associations after correction for multiple
96 comparisons.

97 Increasing quartiles of all trans retinol and lycopene was associated with increased odds of
98 development of rhinitis by 36 months and 60 months albeit without statistical significance (Table
99 3). Contrary to this, increasing quartiles of zeaxanthin and α -cryptoxanthin were associated with
100 decreased odds of rhinitis development in the first 5 years of life albeit without statistical
101 significance.

102 There is a trend of increasing quartiles of zeaxanthin being associated with reduced odds of
103 development of wheeze and use of nebulizer/inhaler in the first 18 months (p-trend= 0.05, table 4).
104 Comparing extreme quartiles, infants in the highest quartile of zeaxanthin had reduced risk of
105 wheezing and use of nebulizer/inhaler by 18 months [adjusted odds ratio 0.3 (0.1-0.96)]. However
106 there were no statistical associations after correction for multiple comparisons.

107 In this study, we found no association between maternal blood levels of carotenoids, vitamin A
108 and vitamin E at delivery with the development of allergen sensitization, eczema, rhinitis and
109 wheeze with use of nebulizer/inhaler in the offspring in first five years of life. To our knowledge,
110 this is the first study with the longest follow-up of children, to have investigated relationships
111 between objective measurements of carotenoids, vitamin A and vitamin E in maternal blood with
112 allergic outcomes in offspring up to five years old. The long follow-up also allows us to evaluate
113 allergic outcomes and respiratory conditions such as wheeze/asthma which has a later age of onset.

114 In agreement with our observations, a study conducted in Poland which included 252 mother-child
115 pairs reported no associations between β -carotene levels in maternal plasma in the first trimester of
116 pregnancy, at delivery and in cord blood with risk of development of atopic dermatitis and wheeze
117 in the first 2 years of life in the offspring [4]. Other longitudinal studies done in mother-child
118 dyads, which used food frequency questionnaires to record β -carotene intake, have also found no
119 association between β -carotene and allergen sensitization [5], eczema [5-8], rhinitis [6] and
120 wheeze [5]. Contrary to our findings, one Japanese study reported negative association between β -
121 carotene intake during pregnancy and eczema in children up to two years old [9]. However,
122 estimates of maternal intake of β -carotene during pregnancy were assessed with a diet history
123 questionnaire and information of multivitamin supplements was not considered in this study.
124 Therefore, the maternal daily intake estimated may not reflect the level of β -carotene accurately.

125 Similar to our findings, two other longitudinal studies in Poland also found no relationships
126 between vitamin A levels measured from maternal blood or cord blood with wheeze in offspring

127 aged one to two years old [4, 10]. Another longitudinal study in mother-child dyads which used
128 food-frequency-questionnaires to record vitamin A intakes also reported no associations with
129 eczema and rhinitis [6].

130 Apart from measuring vitamin A levels, the two Polish studies [4, 10] also investigated
131 relationships between vitamin E levels measured in maternal blood or cord blood and found no
132 associations with wheeze in young offspring, which is consistent with our null finding. Most of the
133 other studies in mother-child dyads used food-frequency-questionnaires to record vitamin E intake
134 and reported no associations between vitamin E and allergic outcomes [5, 6]. Contrary to our
135 findings, two longitudinal studies in mother-child dyads which used food-frequency-
136 questionnaires to record vitamin E intakes showed negative association between vitamin E intake
137 and wheeze [7] and between vitamin E intake and eczema as well as wheeze [8]. However,
138 estimates of maternal intake of vitamin E during pregnancy were assessed with food frequency
139 questionnaires in these two studies. Therefore, the maternal intake estimated may not reflect the
140 actual level of vitamin E accurately.

141 Although without statistical significance, we found a trend of increasing quartiles of zeaxanthin
142 being associated with reduced odds of development of rhinitis and wheezing. Interestingly,
143 another study [7] has shown a protective effect of maternal intake of lutein and zeaxanthin on
144 childhood respiratory infection. As much of early-life wheezing is due to respiratory infections, it
145 is possible that zeaxanthin exerts a protective effect on wheezing by preventing respiratory
146 infections in children.

147 The strengths of this study are the objective measurement of carotenoids, vitamin A and vitamin E
148 in maternal plasma which are not subjected to recall bias. In addition, measurement of plasma
149 carotenoids is reported to be reliable estimates of diet since they are not regulated by homeostasis
150 and are predominantly obtained from the diet [11]. Besides this, there are very regular follow up of
151 children in this study with objective assessment of allergen sensitization by skin prick testing.
152 However, we acknowledge that there are limitations, parental reporting of allergic outcomes may
153 be subjected to recall and reporting bias. The baby's immune development occurs most rapidly
154 during second to third trimester, suggesting that vitamins during pregnancy has to be timely to
155 influence the child's development, hence a single measurement of maternal nutrient levels at
156 delivery may not be an accurate reflection of the nutrient levels throughout pregnancy or the

157 nutrient levels of their offspring. In addition, as we assessed maternal carotenoids at delivery with
158 allergic outcomes over 5 years, other factors such as child's diet will also modulate this effect.

159 In conclusion, we do not observe any associations between maternal carotenoids, vitamin E and
160 vitamin A levels with the development of allergic diseases in the offspring in the first 5 years of
161 life.

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201 **Competing interests**

202 Chong YS has received reimbursement for speaking at conferences sponsored by Abbott
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260

Allergen sensitization		18 Months (N= 605)				36 Months (N=632)					60 Months (N= 553)				
		Adjusted OR (95%CI)				Adjusted OR (95% CI)					Adjusted OR (95% CI)				
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	p-trend	Quartile 1	Quartile 2	Quartile 3	Quartile 4	p-trend	Quartile 1	Quartile 2	Quartile 3	Quartile 4	p-trend
Vitamin A	Ref	1.1 (0.5-2.6)	1.5 (0.6-3.4)	0.8 (0.3-2.0)	0.9	Ref	1.0 (0.5-2.0)	1.2(0.6-2.3)	0.8 (0.4-1.7)	0.7	Ref	1.1 (0.5-2.2)	1.8 (0.9-3.6)	1.0 (0.5-2.1)	0.6
All trans retinol															
Carotenoids	Ref	1.2 (0.5-2.9)	1.4 (0.5-3.9)	1.4 (0.5-4.0)	0.5	Ref	1.2 (0.6-2.4)	2.0 (0.9-4.7)	2.2 (0.9-5.3)	0.048	Ref	1.6 (0.8-3.3)	1.4 (0.6-3.1)	1.4 (0.6-3.3)	0.6
Lutein															
Zeaxanthin	Ref	1.1 (0.5-2.5)	0.9 (0.4-2.2)	0.7 (0.3-1.8)	0.4	Ref	1.5 (0.8-3.1)	1.9 (0.9-4.0)	0.9 (0.4-2.0)	0.98	Ref	0.8 (0.4-1.7)	1.6 (0.8-3.3)	0.8 (0.4-1.7)	0.96
α -cryptoxanthin	Ref	1.5 (0.7-3.5)	0.9 (0.4-2.2)	1.3 (0.5-3.4)	0.9	Ref	1.5 (0.8-3.0)	1.1 (0.5-2.2)	1.1 (0.5-2.4)	0.9	Ref	2.2 (1.1-4.4)	1.3 (0.6-2.6)	1.2 (0.6-2.7)	0.97
β -cryptoxanthin	Ref	1.4 (0.6-3.2)	1.0 (0.4-2.7)	1.4 (0.6-3.5)	0.6	Ref	1.2 (0.6-2.5)	0.7 (0.3-1.6)	1.1 (0.5-2.2)	0.8	Ref	0.9 (0.5-1.9)	0.7 (0.3-1.6)	0.9 (0.5-1.9)	0.7
Lycopene	Ref	0.5 (0.2-1.2)	1.2 (0.5-2.8)	0.7 (0.3-1.7)	0.9	Ref	0.6 (0.3-1.4)	2.3 (1.2-4.7)	1.3 (0.6-2.8)	0.06	Ref	0.4 (0.2-0.9)	0.7 (0.4-1.5)	0.7 (0.3-1.4)	0.6
α -carotene	Ref	1.0 (0.4-2.3)	0.9 (0.4-2.3)	1.5 (0.6-3.8)	0.4	Ref	1.0 (0.5-2.2)	1.7 (0.8-3.7)	1.7 (0.8-3.6)	0.1	Ref	1.1 (0.5-2.2)	1.1 (0.5-2.3)	1.7 (0.8-3.5)	0.2
β -carotene	Ref	1.3 (0.6-3.0)	1.0 (0.4-2.4)	1.5 (0.6-3.8)	0.5	Ref	1.1 (0.5-2.3)	0.8 (0.4-1.7)	2.0 (0.9-4.2)	0.1	Ref	0.8 (0.4-1.7)	1.1 (0.5-2.4)	1.2 (0.6-2.6)	0.5
Vitamin E	Ref	0.7 (0.3-1.5)	0.8 (0.3-1.8)	0.3 (0.1-0.9)	0.05	Ref	1.0 (0.5-2.0)	0.8 (0.4-1.6)	1.1 (0.5-2.2)	0.98	Ref	1.0 (0.5-2.0)	0.9 (0.5-1.9)	0.8 (0.4-1.6)	0.5
γ -tocotrienol															
α -tocotrienol	Ref	0.7 (0.3-1.6)	0.7 (0.3-1.5)	0.3 (0.1-0.8)	0.03	Ref	0.7 (0.4-1.4)	0.7 (0.4-1.4)	0.8 (0.4-1.6)	0.4	Ref	1.2 (0.6-2.3)	0.8 (0.4-1.6)	1.1 (0.6-2.3)	0.97
δ -tocopherol	Ref	0.9 (0.4-2.1)	0.5 (0.2-1.3)	0.8 (0.3-1.9)	0.4	Ref	0.5 (0.2-1.2)	0.9 (0.5-1.9)	1.0 (0.5-2.1)	0.7	Ref	0.6 (0.3-1.2)	0.9 (0.4-1.8)	1.0 (0.5-2.1)	0.7
γ -tocopherol	Ref	1.4 (0.6-3.3)	0.6 (0.3-1.6)	1.0 (0.4-2.5)	0.6	Ref	1.6 (0.8-3.1)	0.7 (0.3-1.6)	0.9 (0.4-2.0)	0.3	Ref	1.7 (0.8-3.4)	0.7 (0.3-1.6)	0.7 (0.3-1.6)	0.1

							3.2)	1.4)	1.8)			3.4)	1.5)	1.4)	
α -tocopherol	Ref	1.1 (0.5-2.7)	1.0 (0.4-2.4)	1.4 (0.6-3.3)	0.5	Ref	2.2 (1.1-4.7)	1.5 (0.7-3.3)	2.1(1.0-4.3)	0.1	Ref	1.4 (0.7-2.9)	1.0 (0.5-2.1)	1.8 (0.9-3.6)	0.2

Table 1: Association between maternal carotenoids, vitamin A, vitamin E levels, and allergen sensitization in offspring in 5 years of life

*Adjusted for gender, ethnicity, maternal age, maternal BMI during pregnancy, maternal education levels, multivitamin use during pregnancy and family history of allergy

Eczema		18 Months (N= 648)				36 Months (N=609)					60 Months (N= 616)				
		Adjusted OR (95%CI)				Adjusted OR (95% CI)					Adjusted OR (95% CI)				
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	p-trend	Quartile 1	Quartile 2	Quartile 3	Quartile 4	p-trend	Quartile 1	Quartile 2	Quartile 3	Quartile 4	p-trend
Vitamin A	Ref	1.3 (0.6-2.6)	0.6 (0.3-1.3)	0.7 (0.3-1.5)	0.1	Ref	1.1 (0.5-2.1)	0.6 (0.3-1.3)	0.6 (0.3-1.2)	0.06	Ref	1.0 (0.5-1.9)	0.6 (0.3-1.2)	0.6 (0.3-1.2)	0.07
All trans retinol															
Carotenoids	Ref	1.5 (0.6-3.2)	1.0 (0.4-2.4)	0.8 (0.3-2.0)	0.4	Ref	1.2 (0.6-2.6)	0.9 (0.4-2.0)	0.7 (0.3-1.7)	0.3	Ref	1.1 (0.5-2.2)	0.8 (0.4-1.9)	0.7 (0.3-1.7)	0.4
Lutein															
Zeaxanthin	Ref	2.2 (1.1-4.5)	1.4 (0.6-3.1)	0.8 (0.3-1.9)	0.5	Ref	1.8 (0.9-3.5)	1.3 (0.6-2.8)	0.9 (0.4-2.1)	0.8	Ref	1.8 (0.9-3.5)	1.3 (0.6-2.8)	0.8 (0.4-1.9)	0.6
α -cryptoxanthin	Ref	1.1 (0.5-2.3)	0.7 (0.3-1.6)	0.7 (0.3-1.5)	0.2	Ref	1.1 (0.5-2.2)	0.8 (0.4-1.6)	0.8 (0.4-1.7)	0.4	Ref	0.9 (0.5-1.9)	0.6 (0.3-1.3)	0.7 (0.3-1.5)	0.2
β -cryptoxanthin	Ref	1.2 (0.5-2.6)	1.2 (0.5-2.8)	1.4 (0.6-3.0)	0.4	Ref	1.3 (0.6-2.8)	1.8 (0.8-3.8)	1.4 (0.6-2.8)	0.3	Ref	1.4 (0.7-2.9)	1.7 (0.8-3.6)	1.4 (0.7-2.9)	0.4
Lycopene	Ref	1.5 (0.7-3.4)	2.3 (1.0-5.1)	1.7 (0.7-4.0)	0.1	Ref	1.6 (0.7-3.5)	2.4 (1.1-5.1)	1.6 (0.7-3.5)	0.2	Ref	1.8 (0.9-4.0)	2.3 (1.1-4.8)	1.6 (0.7-3.5)	0.3
α -carotene	Ref	1.7 (0.8-3.8)	1.1 (0.5-2.6)	0.9 (0.4-2.0)	0.4	Ref	1.7 (0.8-3.7)	1.1 (0.5-2.5)	0.9 (0.4-2.1)	0.5	Ref	1.5 (0.7-3.2)	1.1 (0.5-2.3)	0.9 (0.4-2.1)	0.6
β -carotene	Ref	2.5 (1.1-5.6)	1.1 (0.5-2.5)	1.0 (0.4-2.5)	0.5	Ref	2.3 (1.1-4.9)	0.9 (0.4-2.0)	1.0 (0.4-2.3)	0.5	Ref	2.0 (1.0-4.2)	0.8 (0.4-1.8)	1.0 (0.4-2.1)	0.4
Vitamin E	Ref	0.7 (0.3-1.4)	1.3 (0.6-2.8)	0.9 (0.4-1.9)	0.8	Ref	0.8 (0.4-1.6)	1.2 (0.6-2.5)	1.0 (0.5-2.0)	0.8	Ref	1.0 (0.5-2.0)	1.4 (0.7-3.0)	1.0 (0.5-2.0)	0.8
γ -tocotrienol															
α -tocotrienol	Ref	1.5 (0.7-3.1)	1.4 (0.7-2.9)	1.1 (0.5-2.5)	0.7	Ref	1.6 (0.8-3.2)	1.4 (0.7-2.7)	1.0 (0.4-2.1)	0.97	Ref	1.7 (0.8-3.2)	1.3 (0.7-2.5)	0.9 (0.4-1.9)	0.8
δ -tocopherol	Ref	0.6 (0.3-1.3)	0.8 (0.4-1.6)	0.4 (0.2-0.98)	0.08	Ref	0.6 (0.3-1.3)	1.0 (0.5-2.0)	0.5 (0.2-1.0)	0.2	Ref	0.5 (0.3-1.1)	1.0 (0.5-2.0)	0.5 (0.2-1.0)	0.2
γ -tocopherol	Ref	1.2 (0.6-2.6)	1.0 (0.5-2.1)	0.6 (0.2-1.3)	0.1	Ref	1.2 (0.6-2.6)	0.9 (0.4-2.0)	0.5 (0.3-1.0)	0.09	Ref	0.9 (0.5-1.5)	0.8 (0.4-1.4)	0.5 (0.2-1.0)	0.06

							2.4)	1.9)	1.2)			1.9)	1.6)	1.0)	
α -tocopherol	Ref	1.0 (0.5-2.2)	0.7 (0.3-1.4)	0.8 (0.4-1.6)	0.3	Ref	0.9 (0.5-1.9)	0.6 (0.3-1.3)	0.6 (0.3-1.3)	0.1	Ref	0.9 (0.5-1.9)	0.8 (0.4-1.6)	0.5 (0.2-1.0)	0.06

Table 2: Association between maternal carotenoids, vitamin A, vitamin E levels and eczema in offspring in 5 years of life

*Adjusted for gender, ethnicity, maternal age, maternal BMI during pregnancy, maternal education levels, multivitamin use during pregnancy and family history of allergy

Rhinitis		18 Months (N= 499)				36 Months (N=475)					60 Months (N= 478)				
		Adjusted OR (95%CI)				Adjusted OR (95% CI)					Adjusted OR (95% CI)				
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	p-trend	Quartile 1	Quartile 2	Quartile 3	Quartile 4	p-trend	Quartile 1	Quartile 2	Quartile 3	Quartile 4	p-trend
Vitamin A	Ref	1.8 (0.8-4.4)	1.5 (0.6-3.8)	1.9 (0.7-4.8)	0.3	Ref	1.3 (0.6-2.8)	1.4 (0.7-3.1)	1.9 (0.9-4.2)	0.1	Ref	1.4 (0.7-3.0)	1.6 (0.7-3.3)	2.1 (1.0-4.5)	0.07
All trans retinol															
Carotenoids	Ref	0.7 (0.3-1.5)	0.7 (0.3-1.7)	0.5 (0.2-1.3)	0.2	Ref	1.7 (0.8-3.5)	1.3 (0.5-3.1)	1.0 (0.4-2.5)	0.8	Ref	1.4 (0.7-2.9)	1.2 (0.5-2.7)	0.9 (0.4-2.1)	0.7
Lutein															
Zeaxanthin	Ref	1.7 (0.8-3.7)	1.0 (0.4-2.4)	0.6 (0.2-1.5)	0.2	Ref	1.3 (0.7-2.6)	1.4 (0.6-2.9)	0.7 (0.3-1.6)	0.5	Ref	1.3 (0.7-2.6)	1.2 (0.6-2.5)	0.6 (0.3-1.3)	0.2
α -cryptoxanthin	Ref	1.4 (0.6-3.0)	0.8 (0.3-1.8)	0.6 (0.2-1.4)	0.1	Ref	1.2 (0.6-2.4)	0.8 (0.4-1.6)	0.5 (0.2-1.1)	0.06	Ref	1.2 (0.6-2.4)	0.9 (0.5-1.9)	0.5 (0.2-1.0)	0.06
β -cryptoxanthin	Ref	1.4 (0.6-3.3)	1.7 (0.7-4.0)	1.0 (0.4-2.4)	0.97	Ref	1.6 (0.8-3.3)	1.8 (0.8-4.0)	1.2 (0.6-2.7)	0.6	Ref	1.7 (0.8-3.4)	1.5 (0.7-3.2)	1.2 (0.6-2.6)	0.7
Lycopene	Ref	0.8 (0.3-2.1)	1.6 (0.7-3.7)	1.1 (0.4-2.7)	0.5	Ref	1.4 (0.7-3.0)	1.5 (0.7-3.2)	2.0 (0.9-4.6)	0.1	Ref	1.1 (0.5-2.4)	1.4 (0.7-3.0)	2.1 (0.9-4.7)	0.05
α -carotene	Ref	0.9 (0.4-2.0)	0.8 (0.3-2.0)	1.2 (0.5-2.9)	0.8	Ref	0.9 (0.4-1.8)	0.8 (0.3-1.7)	0.9 (0.4-2.0)	0.7	Ref	1.1 (0.5-2.2)	0.9 (0.4-2.0)	0.9 (0.4-2.1)	0.8
β -carotene	Ref	2.9 (1.2-6.7)*	1.1 (0.5-2.8)	1.3 (0.5-3.4)	0.9	Ref	2.2 (1.0-4.6)	1.2 (0.6-2.5)	0.8 (0.3-1.8)	0.4	Ref	2.0 (1.0-4.2)	1.1 (0.5-2.3)	0.7 (0.3-1.6)	0.3
Vitamin E	Ref	1.0 (0.4-2.3)	2.7 (1.1-6.4)	1.1 (0.5-2.7)	0.4	Ref	1.2 (0.6-2.4)	1.9 (0.9-4.1)	1.2 (0.6-2.6)	0.4	Ref	1.3 (0.6-2.6)	2.1 (1.0-4.4)	1.4 (0.7-2.9)	0.2
γ -tocotrienol															
α -tocotrienol	Ref	0.4 (0.2-1.1)	0.9 (0.4-2.0)	1.0 (0.4-2.2)	0.9	Ref	0.6 (0.3-1.2)	0.9 (0.4-1.8)	0.9 (0.4-1.9)	0.9	Ref	0.5 (0.3-1.1)	0.8 (0.4-1.5)	0.9 (0.4-1.9)	0.9
δ -tocopherol	Ref	2.1 (0.8-5.1)	2.1 (0.9-5.3)	1.4 (0.6-3.7)	0.5	Ref	1.7 (0.8-3.7)	1.6 (0.7-3.3)	1.1 (0.5-2.4)	0.9	Ref	1.6 (0.8-3.4)	1.4 (0.7-3.0)	1.0 (0.5-2.2)	0.96
γ -tocopherol	Ref	1.3(0.6-3.2)	1.3 (0.5-3.0)	1.1 (0.5-2.7)	0.9	Ref	0.9 (0.4-1.9)	0.9 (0.4-1.9)	1.1 (0.5-2.4)	0.7	Ref	1.1 (0.5-2.4)	1.0 (0.5-2.0)	1.3 (0.6-2.8)	0.5

							1.8)	2.0)	2.3)			2.2)	2.2)	2.8)	
α -tocopherol	Ref	1.2 (0.5-2.7)	1.7 (0.7-3.9)	0.7 (0.3-1.7)	0.6	Ref	1.1 (0.5-2.3)	2.0 (0.9-4.3)	0.9 (0.4-2.0)	0.7	Ref	1.2 (0.6-2.3)	1.9 (0.9-3.9)	0.9 (0.4-1.9)	0.9

Table 3: Association between maternal carotenoids, vitamin A, vitamin E levels and rhinitis in offspring in 5 years of life

*Adjusted for gender, ethnicity, maternal age, maternal BMI during pregnancy, maternal education levels, multivitamin use during pregnancy and family history of allergy

Wheeze and use nebulizer/inhaler		18 Months (N= 503)				36 Months (N=481)					60 Months (N= 479)				
		Adjusted OR (95%CI)				Adjusted OR (95% CI)					Adjusted OR (95% CI)				
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	p- trend	Quartile 1	Quartile 2	Quartile 3	Quartile 4	p- trend	Quartile 1	Quartile 2	Quartile 3	Quartile 4	p- trend
Vitamin A	Ref	1.8 (0.6- 5.1)	0.5 (0.1-1.8)	1.3 (0.4-4.1)	0.8	Ref	1.9 (0.8- 4.4)	0.7 (0.3- 1.8)	1.2 (0.4- 3.1)	0.6	Ref	1.4 (0.7- 3.1)	0.8 (0.4- 1.9)	1.0 (0.4- 2.3)	0.6
All trans retinol															
Carotenoids	Ref	0.5 (0.2- 1.4)	0.2 (0.1-0.98)	0.4 (0.1-1.6)	0.1	Ref	0.8 (0.4- 1.9)	0.3 (0.1- 0.98)	0.5 (0.2- 1.5)	0.1	Ref	1.0 (0.5- 2.3)	0.7 (0.3- 1.8)	0.9 (0.3- 2.2)	0.6
Lutein															
Zeaxanthin	Ref	0.8 (0.3- 2.1)	0.7 (0.2-2.0)	0.3 (0.1-0.96)	0.05	Ref	1.1 (0.5- 2.8)	1.5 (0.6- 3.8)	0.8 (0.3- 2.2)	0.9	Ref	1.4 (0.6- 3.1)	1.4 (0.6- 3.2)	0.7 (0.3- 1.8)	0.5
α -cryptoxanthin	Ref	1.8 (0.6- 5.0)	0.7 (0.2-2.1)	0.8 (0.2-2.8)	0.3	Ref	1.5 (0.6- 3.5)	0.6 (0.3- 1.6)	0.7 (0.2- 1.8)	0.2	Ref	1.3 (0.6- 2.8)	0.7 (0.3- 1.5)	0.8 (0.3- 1.9)	0.3
β -cryptoxanthin	Ref	1.0 (0.3- 2.9)	1.7 (0.6-5.2)	1.0 (0.3-3.1)	0.8	Ref	1.1 (0.5- 2.7)	1.3 (0.5- 3.1)	0.8 (0.3- 2.0)	0.7	Ref	0.9 (0.4- 2.0)	1.1 (0.5- 2.5)	0.9 (0.4- 2.0)	0.9
Lycopene	Ref	0.8 (0.2- 2.7)	1.3 (0.4-4.2)	1.6 (0.5-5.0)	0.3	Ref	0.7 (0.3- 1.9)	1.0 (0.4- 2.4)	0.9 (0.3- 2.3)	0.99	Ref	1.0 (0.4- 2.5)	1.6 (0.7- 3.8)	1.7 (0.7- 4.1)	0.2
α -carotene	Ref	2.1 (0.8- 5.5)	0.1 (0.02-1.3)	1.2 (0.4-3.9)	0.6	Ref	1.3 (0.6- 2.8)	0.5 (0.2- 1.3)	0.8 (0.3- 2.2)	0.4	Ref	1.5 (0.7- 3.3)	0.7 (0.3- 1.7)	1.5 (0.6- 3.5)	0.8
β -carotene	Ref	1.3 (0.5- 3.4)	0.5 ((0.1-1.9)	0.9 (0.3-2.8)	0.6	Ref	1.6 (0.7- 3.8)	0.7 (0.2- 1.7)	0.7 (0.3- 2.0)	0.3	Ref	1.7 (0.8- 3.8)	0.8 (0.4- 2.0)	1.2 (0.5- 2.8)	0.9
Vitamin E	Ref	0.5 (0.2- 1.5)	1.0 (0.3-2.7)	0.6 (0.2-1.7)	0.5	Ref	0.9 (0.4- 2.1)	1.1 (0.4- 2.9)	1.0 (0.4- 2.5)	0.9	Ref	1.0 (0.4- 2.2)	1.3 (0.6- 3.1)	1.2 (0.5- 2.8)	0.5
γ -tocotrienol															
α -tocotrienol	Ref	0.8 (0.3- 2.2)	0.6 (0.2-1.7)	0.4 (0.1-1.4)	0.1	Ref	0.7 (0.3- 1.7)	0.7 (0.3- 1.7)	0.7 (0.3- 1.7)	0.4	Ref	0.8 (0.4- 1.9)	0.9 (0.4- 1.9)	1.2 (0.5- 2.7)	0.7
δ -tocopherol	Ref	1.6 (0.5- 4.9)	1.6 (0.5-5.1)	0.7 (0.2-2.5)	0.6	Ref	1.4 (0.6- 3.5)	1.0 (0.4- 2.7)	0.9 (0.3- 2.5)	0.7	Ref	1.0 (0.4- 2.3)	1.0 (0.4- 2.2)	0.7 (0.3- 1.6)	0.4
γ -tocopherol	Ref	1.1 (0.4- 3.1)	0.4 (0.1-1.5)	1.4 (0.5-3.9)	0.9	Ref	1.0 (0.4- 2.5)	0.8 (0.3- 2.0)	0.9 (0.4- 2.3)	0.8	Ref	1.3 (0.6- 2.8)	1.0 (0.4- 2.2)	0.7 (0.3- 1.6)	0.4

		3.2)					2.3)	2.1)	2.3)			2.9)	2.3)	1.7)	
α -tocopherol	Ref	1.6 (0.5-4.6)	2.0 (0.7-5.8)	0.5 (0.1-1.9)	0.5	Ref	1.7 (0.7-4.1)	1.9 (0.8-4.8)	0.9 (0.3-2.3)	0.9	Ref	1.4 (0.6-3.0)	1.7 (0.8-3.9)	0.7 (0.3-1.6)	0.5

Table 4: Association between maternal carotenoids, vitamin A, vitamin E levels and wheeze with nebulizer/inhaler in offspring in 5 years of life

*Adjusted for gender, ethnicity, maternal age, maternal BMI during pregnancy, maternal education levels, multivitamin use during pregnancy and family history of allergy

Supplementary Table 1

	Study population	Excluded from analysis	p-value
	N(%)	N (%)	
Sex			
Male	372 (53.1)	247 (52.4)	0.9
Female	329 (46.9)	224 (47.6)	
Ethnicity			
Chinese	410 (58.5)	281 (52.5)	0.06
Malay	166 (23.7)	157 (29.3)	
Indian	125 (17.8)	97 (18.1)	
Mode of delivery			
Vaginal	424 (63.3)	300 (66.1)	0.3
Cesarean	246 (36.7)	154 (33.9)	
Maternal education levels			
Less than 12 years	270 (38.8)	235 (44.9)	0.03
At least 12 years	426 (61.2)	288 (55.1)	
Family history of allergy	250 (50.6)	139 (49.5)	0.8
No family history of allergy	244 (49.4)	142 (50.5)	

Table 1 : Demographics of study population

Supplementary methods :

We used the ISAAC modified questionnaire as used in other studies. Study team members called the subjects who reported rhinitis to collect information on the number of episodes of rhinitis and the duration of each episode. A case prior to 18 months required a single episode that lasted for at least 4 weeks or two or more episodes each lasting at least 2 weeks. New cases of rhinitis after 18 months were defined by one or more episodes lasting at least 2 weeks.

For allergic outcomes (eczema, rhinitis, wheeze and use of nebulizer) by 18 months, the allergic outcome was classified as absent when the answers for the visits and/or at 18 month were “no.” For allergic outcomes (eczema, rhinitis, wheeze and use of nebulizer) by 36 months, the allergic outcome was classified as absent when the answers for the visits were “no” for the first 18 months and subsequent time points. For allergic outcomes (eczema, rhinitis, wheeze and use of nebulizer) by 60 months, the allergic outcome was classified as absent when the answers for the visits were “no” for the first 18 months and at least 75% of subsequent timepoints.

The UPLC method is as follows: in an amber micro-centrifuge tube, a 30 μ L aliquot of plasma was deproteinized with equal volume of EB solution (ethanol-tert-butanol, 4:1, v/v) and I.S. (echinenone, 0.4 mg/L). It was then extracted with 100 μ L of n-hexane for 2 min. After centrifugation (15 000 g/ 1 min), 160 μ L of supernatant was transferred into another amber micro-centrifuge tube and dried under a stream of nitrogen. The dried residue was reconstituted in 60 μ L of EB solution and 5 μ L was injected onto a Kinetex C18 core-shell (2.6 μ m, 100 mm x 4.6 mm ID; Phenomenex). The four mobile phase solutions used for gradient separation were: A, pure acetonitrile; B, pure methanol; and C, a mixture of ethanol and tert-butanol (8:2, v/v) and D, pure water. Using a Waters Acquity H-class UPLC system, the gradient separation was initiated with 100% D at a constant flow rate of 0.6 ml/min and

linearly changed to 100% B within 0.1 min, 10% A and 90% B from 0.1-6 min, 40% A and 60% C from 6-8 min and 100% C from 10-14 min. The column was then re-equilibrated with water (100% D) for 5min before the next injection of sample.

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Male	372 (53.1)	247 (52.4)	0.9
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At least 12 years	426 (61.2)	288 (55.1)	
Family history of allergy	250 (50.6)	139 (49.5)	0.8
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Table 1 : Demographics of study population

Supplementary methods :

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For allergic outcomes (eczema, rhinitis, wheeze and use of nebulizer) by 18 months, the allergic outcome was classified as absent when the answers for the visits and/or at 18 month were “no.”

For allergic outcomes (eczema, rhinitis, wheeze and use of nebulizer) by 36 months, the allergic outcome was classified as absent when the answers for the visits were “no” for the first 18 months and subsequent time points. For allergic outcomes (eczema, rhinitis, wheeze and use of nebulizer) by 60 months, the allergic outcome was classified as absent when the answers for the visits were “no” for the first 18 months and at least 75% of subsequent timepoints.

The UPLC method is as follows: in an amber micro-centrifuge tube, a 30 μ L aliquot of plasma was deproteinized with equal volume of EB solution (ethanol-tert-butanol, 4:1, v/v) and I.S. (echinenone, 0.4 mg/L). It was then extracted with 100 μ L of n-hexane for 2 min. After centrifugation (15 000 g/ 1 min), 160 μ L of supernatant was transferred into another amber micro-centrifuge tube and dried under a stream of nitrogen. The dried residue was reconstituted in 60 μ L of EB solution and 5 μ L was injected onto a Kinetex C18 core-shell (2.6 μ m, 100 mm x 4.6 mm ID; Phenomenex). The four mobile phase solutions used for gradient separation were: A, pure acetonitrile; B, pure methanol; and C, a mixture of ethanol and tert-butanol (8:2, v/v) and D, pure water. Using a Waters Acquity H-class UPLC system, the gradient separation was initiated with 100% D at a constant flow rate of 0.6 ml/min and linearly changed to 100% B within 0.1 min, 10% A and 90% B from 0.1-6 min, 40% A and 60% C from 6-8 min and 100%

C from 10-14 min. The column was then re-equilibrated with water (100% D) for 5min before the next injection of sample.