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

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**To the Editor,**

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

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

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

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

Allergen sensitization was determined by skin prick testing (SPT). SPT to inhalant allergens (house dust mites *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae,* and *Blomia tropicalis*) and food allergens (egg, peanut and cow’s milk) was carried out at 18, 36 and 60 months. At 60 months, SPT was also carried out to shrimp and crab allergens. All tests were interpreted as positive if the wheal was at least 3 mm, and a child was considered as SPT-positive if any one or more of the individual tests was positive with a positive reaction to the positive control (histamine) and a negative reaction to the negative control (saline).

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

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

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

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

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

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

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

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

Similar to our findings, two other longitudinal studies in Poland also found no relationships between vitamin A levels measured from maternal blood or cord blood with wheeze in offspring aged one to two years old [4, 10]. Another longitudinal study in mother-child dyads which used food-frequency-questionnaires to record vitamin A intakes also reported no associations with eczema and rhinitis [6].

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

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

The strengths of this study are the objective measurement of carotenoids, vitamin A and vitamin E in maternal plasma which are not subjected to recall bias. In addition, measurement of plasma carotenoids is reported to be reliable estimates of diet since they are not regulated by homeostasis and are predominantly obtained from the diet [11]. Besides this, there are very regular follow up of children in this study with objective assessment of allergen sensitization by skin prick testing. However, we acknowledge that there are limitations, parental reporting of allergic outcomes may be subjected to recall and reporting bias. The baby’s immune development occurs most rapidly during second to third trimester, suggesting that vitamins during pregnancy has to be timely to influence the child’s development, hence a single measurement of maternal nutrient levels at delivery may not be an accurate reflection of the nutrient levels throughout pregnancy or the nutrient levels of their offspring. In addition, as we assessed maternal carotenoids at delivery with allergic outcomes over 5 years, other factors such as child’s diet will also modulate this effect.

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

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**Competing interests**

Chong YS has received reimbursement for speaking at conferences sponsored by Abbott Nutrition, Nestle, and Danone. Godfrey KM has received reimbursement for speaking at conferences sponsored by Nestle and Shek LP has received reimbursement for speaking at conferences sponsored by Danone and Nestle and consulting for Mead Johnson and Nestle.

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| --- | --- | --- | --- | --- |
| **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 (0.8-3.2) | 0.7 (0.3-1.4) | 0.9 (0.4-1.8) | 0.3 | Ref | 1.7 (0.8-3.4) | 0.7 (0.3-1.5) | 0.7 (0.3-1.4) | 0.1 |
| 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 (0.6-2.4) | 0.9 (0.4-1.9) | 0.5 (0.3-1.2) | 0.09 | Ref | 0.9 (0.5-1.9) | 0.8 (0.4-1.6) | 0.5 (0.2-1.0) | 0.06 |
| 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 (0.4-1.8) | 0.9 (0.4-2.0) | 1.1 (0.5-2.3) | 0.7 | Ref | 1.1 (0.5-2.2) | 1.0 (0.5-2.2) | 1.3 (0.6-2.8) | 0.5 |
| 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 (0.4-3.2) | 0.4 (0.1-1.5) | 1.4 (0.5-3.9) | 0.9 | Ref | 1.0 (0.4-2.3) | 0.8 (0.3-2.1) | 0.9 (0.4-2.3) | 0.8 | Ref | 1.3 (0.6-2.9) | 1.0 (0.4-2.3) | 0.7 (0.3-1.7) | 0.4 |
| 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