Delineation of the Individual Effects of Vitamin E Isoforms on Early Life Incident Wheezing

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Objectives To test the hypothesis that maternal plasma alpha-tocopherol levels are associated with protection from childhood wheeze and that this protection is modified by gamma-tocopherol.

Study design We conducted a prospective nested study in the Infant Susceptibility to Pulmonary Infections and Asthma Following Respiratory Syncytial Virus Exposure birth cohort of 652 children with postpartum maternal plasma vitamin E isoforms used as a surrogate for pregnancy concentrations. Our outcomes were wheezing and recurrent wheezing over a 2-year period, ascertained using validated questionnaires. We assessed the association of alpha- and gamma-tocopherol with wheezing outcomes using multivariable adjusted logistic regression, and tested for interaction between the isoforms with respect to the risk for wheezing outcomes.

Results Children with wheezing (n = 547, n = 167; 31%) and recurrent wheezing (n = 545, n = 55; 10.1%) over a 2-year period were born to mothers with significantly lower postpartum maternal plasma concentrations of alpha-tocopherol, $P = .016$ and $P = .007$, respectively. In analyses of IQR increases, alpha-tocopherol was associated with decreased risk of wheezing (aOR 0.70 [95% CI 0.53,0.92]) and recurrent wheezing (aOR 0.63 [95% CI 0.42,0.95]). For gamma-tocopherol, the aOR for wheezing was 0.79 (95% CI 0.56-1.10) and the aOR for recurrent wheezing was 0.56 (95% CI 0.33-0.94, with nonmonotonic association). The association of alpha-tocopherol with wheezing was modified by gamma-tocopherol (P interaction = .05).

Conclusions Increases in postpartum maternal plasma alpha-tocopherol isoform concentrations were associated with decreased likelihood of wheezing over a 2-year period. Gamma-tocopherol modified this association. *(J Pediatr 2019;206:156-63).*

Asthma, characterized by airway inflammation, oxidative stress, and airway hyper-reactivity, is one of the most common chronic childhood diseases worldwide, with approximately 80% of disease onset during childhood.[1-5] Marked variation in asthma rates around the world,[6] rapid increases in population-specific rates of asthma over time as countries develop in a westernized fashion,[7] and shifts in risk of asthma for migrating populations toward that of their adoptive country[8] suggests that the environment, not genes alone, is likely a significant contributor to disease development. There are currently no effective interventions to prevent childhood asthma, but these observations suggest that the ability to modify diet, environmental exposures, and lifestyle are likely to prevent disease if causal early life and modifiable risk factors can be identified.

Dietary interventions for asthma are appealing given the need for safe and cost efficient prevention efforts. Vitamin E is known to be a potent antioxidant as well as a factor affecting cell-mediated immunity, likely important in asthma development[9-11] and lung growth.[12,13] Vitamin E is a fat soluble vitamin requiring dietary intake in humans. Ninety-nine percent of body vitamin E is estimated to reside in the tissues, especially in slowly turned over body stores (likely in the adipose tissue) that maintain steady state plasma concentrations.[14,15] Plasma concentrations do not fluctuate much because of this reservoir.[14,16] Vitamin E concentrations in the plasma can be increased by absorption of dietary vitamin E, but are mostly maintained by recirculation from the tissues.[15] Short courses of exogenous

ARI	Acute respiratory illness
BMI	Body mass index
HRV	Human rhinovirus
INSPIRE	Infant Susceptibility to Pulmonary Infections and Asthma Following Respiratory Syncytial Virus Exposure
PCR	Polymerase chain reaction
RSS	Respiratory severity score
RSV	Respiratory syncytial virus

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supplementation of vitamin E produce short-lived increases in plasma concentrations[17]; whereas, increases in tissue concentrations, where vitamin E is exerting its effects, require long-term modification of vitamin E intake.[18] It is estimated that >90% of American adults do not meet the estimated average requirement of 12 mg/day of α-tocopherol.[19] During pregnancy, maternal vitamin E plasma concentrations in women who are not taking vitamin E supplements gradually increase until delivery and decrease during the postpartum period.[16,20]

We have previously demonstrated a protective effect of the vitamin E isoform, alpha-tocopherol, on incident adult-onset asthma, as well as an opposing effect of the vitamin E isoform, gamma-tocopherol, at higher concentrations.[9] Although all isoforms of vitamin E are known to scavenge free radicals,[21] we have demonstrated in in vivo mouse models of allergic inflammation that individual isoforms of vitamin E have different effects.[10,22-24] Alpha-tocopherol, the most abundant form of vitamin E in the body, reduced allergic airway inflammation to allergen challenge for the offspring of female allergic mice in a dose-dependent fashion.[23] In contrast, gamma-tocopherol supplementation in the same mouse model increased allergic airway responses but has been demonstrated to reduce neutrophilic airway inflammation in other models.[24-29] As the ratio of these isoforms varies by oil source consumed, dietary modification favoring one isoform over another is both possible and inexpensive.[22]

In a recent systematic review and meta-analysis, maternal vitamin E was associated with reduced subsequent wheezing in childhood.[30] To date, the independent associations and interaction of both major vitamin E isoforms, alpha-tocopherol, and gamma-tocopherol, on the development of pediatric wheezing illnesses have not been studied. The objective of this study was to assess the relationships of maternal vitamin E isoforms on childhood wheezing.[31,32]

Methods

The Infant Susceptibility to Pulmonary Infections and Asthma Following Respiratory Syncytial Virus (RSV) Exposure (INSPIRE) study is a prospective, ongoing birth cohort of 1951 healthy term infants enrolled during the first few months of infancy designed to investigate the association of early life environmental exposures with the development of allergic and respiratory outcomes. Methods for the INSPIRE birth cohort have previously been published, (overview in the **Appendix**; available at www.jpeds.com).[33] In brief, baseline interviews, sample collections, and standardized questionnaires were conducted at enrollment within the first few months of the infant's birth. Follow-up for the outcomes of wheezing, asthma and allergic diseases is ongoing annually using validated instruments including the International Study of Asthma and Allergies in Childhood questionnaire.[34]

The current study includes 652 mother-child dyads with available postpartum maternal blood samples from the subset of 1090 participants who were approached to provide a maternal blood sample (**Figure 1**; available at www.jpeds.com). Written informed consent was obtained from the parent at enrollment. The study was approved by the Institutional Review Board at Vanderbilt University Medical Center. Data were collected and managed using the REDCap tool hosted at Vanderbilt University (Nashville, Tennessee).[35]

Maternal Alpha- and Gamma-Tocopherol Concentrations

During the second recruitment phase of INSPIRE postpartum period enrollment encounters, we obtained a fingerstick blood sample on a subset of 652 mothers (59.8% of second recruitment cohort). Postpartum levels of vitamin E have been shown to slowly decrease after pregnancy, and the stability of vitamin E plasma concentrations over time supports the acceptability of a single time point measure as a surrogate for pregnancy concentrations.[14,16] Plasma levels of alpha- and gamma-tocopherol were assessed using high performance liquid chromatography per a previously published protocol.[36,37]

Childhood Respiratory Outcomes

Our primary childhood respiratory outcome, which we will refer to as wheezing over a 2-year period, throughout this report, was defined at age 2 years as wheezing in the past 12 months, or receipt of asthma medications in the past 12 months or a parent reported diagnosis of asthma. To capture more severe wheezing, our second childhood respiratory outcome was recurrent wheezing, defined as ≥3 episodes of wheezing between the child's first birthday and the 2-year visit. A detailed overview of the outcome definitions can be found in the **Appendix**.

Statistical Analyses

Demographic and other baseline characteristics were described for the nested cohort (n = 652) using median and IQR for continuous variables; frequencies and proportions were used for categorical variables and were compared using Kruskal-Wallis nonparametric test and χ^2 test for overall difference in distribution across the three alpha- and gamma-tocopherol tertiles[9] (**Table I** and **Table II**; available at www.jpeds.com)

We assessed the relationship of alpha-tocopherol and gamma-tocopherol on wheezing and recurrent wheezing over a 2-year period, using univariate analyses and multivariable logistic regression. We also tested for interaction between the 2 vitamin isoforms to examine whether the relationship of alpha-tocopherol with wheezing risk was modified by gamma-tocopherol, as we have shown in adults with asthma.[9] Vitamin E isoforms were analyzed with log transformation or restricted cubic splines with 3 knots when nonlinear associations were detected (as for gamma-tocopherol). To depict interaction association, tertiles of maternal gamma-tocopherol isoforms were used for ease of interpretation in plotting the continuous relationship of alpha-tocopherol and child wheezing outcome. Maternal gamma-tocopherol levels were imputed to 0.01 (n = 4) when log transformation was necessary.

Covariates were selected a priori for their known relationship with wheezing, recurrent wheezing, or impact on host antioxidant defense and included infant sex, race, birth weight

(g), breastfeeding, maternal smoking during pregnancy, maternal asthma, and maternal insurance status. Postpregnancy maternal body mass index (BMI, kg/m^2) was missing in ~20% of the subcohort, thus, to assess the impact of case wise deletions when it was included as a covariate for adjustment, we performed sensitivity analysis with multiple imputations. We controlled for potential differences in the timing of postpartum maternal vitamin E isoform measurement by performing sensitivity analyses with infant age at enrollment using restricted cubic splines to account for nonlinear associations. Although we have a term birth cohort, to control for potential residual confounding, we performed a separate analysis with wheezing over a 2-year period and infant gestational age as an additional covariate in multivariable regression. Separate analyses were conducted with adjustment for RSV positive or human rhinovirus (HRV) positive status during acute upper respiratory infection in infancy (both assessed by polymerase chain reaction) along with the main a priori covariates listed above.

A 2-sided 5% significance level was used for all statistical inferences. Statistical analyses were performed using R v 3.4.0 (R Foundation, Vienna, Austria).[38]

Results

Our study population is comprised of 652 mother-child dyads from the INSPIRE cohort with available maternal tocopherol isoform concentrations. The subset cohort reflects the demographics of the local population, with a proportion of race and ethnicities reflective of urban Davidson County and suburban and rural Williamson, Sumner Counties in Tennessee, from which the study population is derived[39] (**Table III** and **Table IV** [available at www.jpeds.com]). Children who had mothers with available serum tocopherol isoform concentrations for study were not significantly different from those in the rest of the INSPIRE cohort in regard to infant sex, birth weight, gestational age, prenatal vitamin use, breastfeeding, type of insurance, or maternal BMI (data not shown). Cohort characteristics were compared by tertiles of both alpha and gamma-tocopherol (**Table I** and **Table II**).

Alpha-tocopherol concentrations in our population ranged from a minimum level of 4.2 μmol/L to a maximum of 217.8 μmol/L, with a median of 73.1 μmol/L (IQR: 46.1,102.3) (**Figure 2**; available at www.jpeds.com). Only 4 mothers were deficient in alpha-tocopherol using currently suggested cutoffs for deficiency at 11.6 μmol/L.[21] There is no currently established serum limit to define excess of alpha-tocopherol.

Gamma-tocopherol concentrations in our population ranged from a minimum of 0 μmol/L to a maximum of 51.3 μmol/L, with a median of 8.3 μmol/L (IQR 4.7, 13.2) (**Figure 2**). There are no established serum cutoffs for deficiency or excess of gamma-tocopherol.[21]

Association of Alpha- and Gamma-Tocopherol with Wheezing over a 2-Year Period

In univariate analysis, maternal vitamin E alpha-tocopherol isoform concentrations were significantly lower among children with wheezing over a 2-year period (n = 167 [31%]), median (IQR) of 69 μmol/L (42, 96) compared with children who did not wheeze (n = 380 [69%]) (75 μmol/L [50,106]), $P = .016$. There was no significant difference in maternal gamma-tocopherol isoform concentrations among children who had wheezing over a 2-year period (8.0 μmol/L [4.4, 14.3]) vs children who did not wheeze (8.2 μmol/L [4.7, 12.9]), $P = .92$.

We then assessed the association of maternal tocopherol concentrations with risk of wheezing over a 2-year period using logistic regression. Increasing alpha-tocopherol levels were associated with decreased odds of wheezing over a 2-year period, unadjusted univariate OR 0.70, (95% CI [0.54-0.91]), for IQR difference (46, 102 μmol/L) and aOR 0.70 (95% CI 0.53,0.92). There was no significant association with gamma-tocopherol on wheeze over a 2-year period; unadjusted OR for gamma-tocopherol, 0.83 (95% CI 0.60-1.15) and aOR 0.79 (95% CI 0.56-1.10) for IQR difference (4.7, 13 μmol/L). The relationship of the isoforms with predicted probability of wheezing over a 2-year period was plotted to visualize the relationship between increasing alpha-tocopherol and decreased likelihood of wheezing across the whole range of values. Gamma-tocopherol had a nonmonotonic relationship with risk of wheezing, whereby lower concentrations trended with decreased likelihood of wheezing and higher concentrations with increased likelihood of wheezing (**Figure 3**).

We assessed for the interaction between maternal alpha- and gamma-tocopherol concentrations on the primary outcome of wheezing over a 2-year period with adjustment for potential confounders using multivariable logistic regression. Although increasing maternal alpha-tocopherol concentrations

Table III. Study participants' demographic characteristics

Infants	Maternal vitamin E subcohort (n = 652) Median [IQR: 25th, 75th] or n (%)
Gestational age (wk)	39 [39, 40]
Infant age at enrollment (d)	50 [16, 80]
Infant race or ethnicity	
Black	134 (21%)
White	400 (61%)
Hispanic	64 (10%)
Other	54 (8%)
Birth weight (g)	3433 [3150, 3774]
Sex	
Female	343 (53%)
Male	309 (47%)
Parents	
Maternal age at enrollment (y)	26 [23,31]
Postpregnancy BMI*	27.5 [24.2, 32.4]
Mother ever smoked	240 (37%)
Smoked during pregnancy	115 (18%)
Prenatal vitamin use during pregnancy	609 (93%)
Breastfeeding at enrollment	331 (51%)
Maternal history of asthma	141 (22%)
Maternal history of allergies	171 (26%)
Paternal history of asthma	100 (15%)
Paternal history of allergies	166 (25%)

*BMI (kg/m^2) was available in n = 517 of the vitamin E participants.

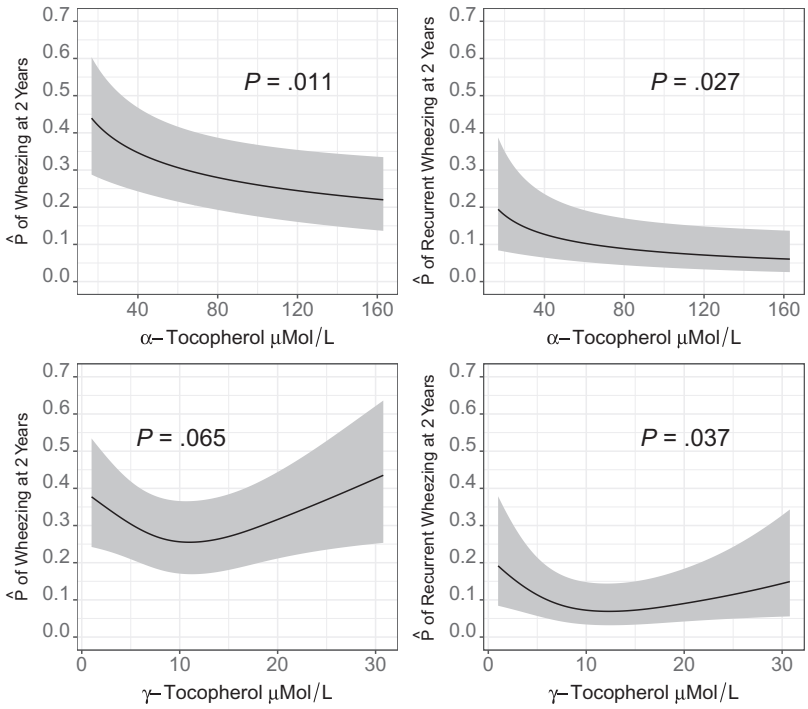

Figure 3. Predicted probability of 2-year wheezing and 2-year recurrent wheezing by maternal alpha- and gamma-tocopherol concentrations. Multiple logistic regression was used to assess the association of alpha- or gamma-tocopherol separately with 2-year wheezing outcomes (2-year wheeze and 2-year recurrent wheeze). Alpha-tocopherol was fit with log transformation, whereas gamma tocopherol showed statistically significant nonlinearity and was fit with restricted cubic splines. Model covariates included infant sex, race, birth weight, breastfeeding status, maternal smoking during pregnancy, maternal asthma, and insurance status. The Y axis in the left two panels represents the adjusted predicted probability of 2-year wheezing, and the Y axis in the right two panels represents the adjusted predicted probability of 2-year recurrent wheezing. The X axis in the panels presents maternal concentrations of alpha-tocopherol (log transformed, $P = .011$ for 2-year wheezing and $P = .027$ for 2-year recurrent wheezing) or gamma-tocopherol ($P = .065$, P nonlinearity = .022 for 2-year recurrent wheezing and $P = .037$, P nonlinearity = .010).

were inversely associated with child wheezing at age 2 years, increasing maternal gamma-tocopherol concentrations appear to attenuate this protective association (P interaction via likelihood ratio test = .05) (**Table V** and **Figure 4**).

When additionally adjusting for maternal postpartum BMI, we performed multiple imputations as 20% of values were missing. In separate models, the association of alpha-tocopherol and gamma-tocopherol remained similarly associated with wheezing over a 2-year period (alpha-tocopherol, $P = .03$; gamma-tocopherol, $P = .06$). In modeling that examined interaction between the 2 isoforms with BMI multiply imputed, gamma tocopherol in tertiles and alpha tocopherol had similar effect modification ($P = .06$).

To control for potential differences because of timing of postpartum maternal gamma- and alpha-tocopherol measurement and residual confounding we also performed additional adjustment for infant age at enrollment and gestational age. In a model containing infant age at enrollment, the association of alpha-tocopherol and gamma-tocopherol remained similarly associated with wheezing over a 2-year period (alpha-tocopherol, $P = .008$; gamma-tocopherol, $P = .08$, P value for interaction = .02). In a model containing both infant age at enrollment and gestational age the association of alpha-tocopherol and gamma-tocopherol also remained similarly associated with wheezing over a 2-year period was unchanged. Neither of these age and timing of exposure measurement-related factors altered the association between maternal alpha- and gamma-tocopherol and wheezing over a 2-year period.

We explored whether the relationship of alpha- and gamma-tocopherol with the subsequent development of childhood wheezing was influenced by infant upper respiratory viral infections by including RSV or HRV detection (polymerase chain reaction confirmed) during acute respiratory infection (ARI) in infancy as an additional covariate in multivariable logistic regressions. Alpha-tocopherol remained associated with wheezing over a 2-year period (OR 0.71, 95% CI 0.54, 0.93, $P = .014$). Adjustment for infant RSV ARI did not attenuate the association of gamma-tocopherol with wheezing over a 2-year period (OR 0.81, 95% CI 0.58, 1.14, $P = .08$). The interaction of alpha-tocopherol with gamma-tocopherol on wheezing over a 2-year period ($P = .05$) remained unchanged with adjustment for RSV ARI in infancy. Similarly, adjustment for HRV detection during an infant ARI did not affect the association between alpha-tocopherol (OR 0.71, 95% CI 0.54, 0.94,

Table V. Interaction between alpha- and gamma-tocopherol on 2-year wheezing

Univariate analysis: Distribution of alpha-tocopherol concentrations by 2-year wheezing status in subjects with low, medium, and high gamma-tocopherol concentrations (tertiles)

		Gamma-tocopherol concentration (tertiles, ng/mg creatinine)		
		Low	Medium	High
Alpha-tocopherol concentration (ng/mg creatinine) [IQR]	No wheezing	49 [36,74]	73 [55,98]	106 [83,123]
Alpha-tocopherol concentration (ng/mg creatinine) [IQR]	Wheezing	42 [26,53]	67 [45,82]	107 [87,121]
P value		0.004	0.059	0.42

Multivariable regression analysis: The protective association of alpha-tocopherol with 2-year wheezing outcome differs by gamma-tocopherol level

		Gamma-tocopherol concentration (tertiles, ng/mg creatinine)		
		Low	Medium	High
Alpha-tocopherol concentration (ng/mg creatinine) IQR difference	Unadjusted ORs*	0.51 (0.32,0.80)	0.54 (0.28,1.05)	1.64 (0.71,3.81)
	aORs*	0.49 (0.30,0.80)	0.59 (0.29,1.18)	1.65 (0.67,4.01)

*ORs were estimated for the association of alpha tocopherol with 2-year wheezing in low, medium, and high tertiles of gamma-tocopherol. Multivariable logistic regression was used for the adjusted association including infant sex, race, birth weight, breastfeeding status, maternal smoking during pregnancy, maternal asthma, and insurance status. *P* interaction (likelihood ratio test) cross-product alpha (continuous)—and gamma-tocopherol tertiles = 0.05. Alpha-tocopherol was natural log transformed.

P = .016) or gamma-tocopherol (OR 0.80, 95% CI 0.57, 1.12, *P* = .065) and wheezing over a 2-year period, nor the interaction between the 2 isoforms (*P* interaction = .06).

Association of Alpha- and Gamma-Tocopherol with Recurrent Wheezing over a 2-Year Period

In univariate analysis, maternal vitamin E alpha-tocopherol isoform concentrations were also significantly lower among mothers of children with recurrent wheezing over a 2-year period (n = 55 [10.1%]), median (IQR) of 53 μmol/L (40, 89) compared with concentrations in mothers of children with less frequent or no wheezing (n = 490 [74.8%]) (75 μmol/L [49,104]), *P* = .007. There was no significant difference in gamma-tocopherol isoform concentrations in mothers of children who had recurrent wheezing over a 2-year period (7.6 μmol/L [4.2, 10.9]) vs mothers of children who did not wheeze (8.3 μmol/L [4.7, 10.9]), *P* = .35.

We then assessed the association of tocopherol concentrations with risk of recurrent wheezing over a 2-year period using logistic regression analysis. Increasing alpha-tocopherol concentrations had a monotonic association with decreased odds of recurrent wheezing over a 2-year period, unadjusted OR 0.60 (95% CI 0.41-0.88), IQR range difference (46, 102 μmol/L), and aOR 0.63 (95% CI 0.42,0.95). Gamma-tocopherol again had a nonmonotonic relationship with risk of recurrent wheezing characterized by protective relationship at lower concentrations and increasing likelihood for recurrent wheezing at higher concentrations, and is best depicted graphically (**Figure 3**). Using difference estimates for IQRs (4.7, 13 μmol/L), the gamma-tocopherol unadjusted OR for recurrent wheezing over a 2-year period was 0.67 (95% CI 0.42-1.07) and aOR 0.56 (95% CI 0.33-0.94).

Discussion

This study demonstrates the important differential relationship of the 2 most prevalent isoforms of vitamin E, alpha- and gamma-tocopherol, on the risk of childhood wheezing. Increasing maternal vitamin E alpha-tocopherol concentrations were associated with decreased odds of childhood wheezing, and increasing gamma-tocopherol concentrations had a nonmonotonic relationship with risk of wheezing and recurrent wheezing. As dietary oils and supplements of vitamin E can contain widely different ratios of both alpha- and gamma-tocopherol, dietary changes or supplementation make vitamin E isoforms a readily modifiable exposure.[22]

Our observations build upon what we and others have reported in studies assessing the relationship of tocopherol isoforms with the development of adult-onset asthma,[9] rate of lung function decline,[40] and adult lung function.[13] An intrauterine effect of alpha-tocopherol on infant and child respiratory morbidity is supported by studies demonstrating associations of increasing maternal alpha-tocopherol concentrations with increased intrauterine crown-rump length in the first trimester, and higher forced expiratory volume at 1 second and forced vital capacity at age 5 years.[41] Biological plausibility is supported by our animal studies demonstrating that selective alpha-tocopherol supplementation of pregnant mice decreases allergic airway inflammation in their offspring, and gamma-tocopherol supplementation potentiates it.[23,24,42] Other animal and human studies have demonstrated potential benefits for gamma-tocopherol on neutrophilic airway inflammation after endotoxin challenge.[25-29] Our study demonstrates the different effects of alpha- and gamma-tocopherol, and potential effect modification of the 2 most common vitamin E isoforms on the outcome of childhood wheezing.

These findings are of significance. First, although previous human studies of self-reported intake of total vitamin E by food frequency questionnaire have sometimes demonstrated a protective effect of vitamin E intake on asthma development, the alpha- and gamma-tocopherol content can vary widely.[43,44] The differential effect of these isoforms may explain why these findings have been inconsistent, especially in comparing studies done in regions where dietary sources of vitamin E, and, thus,

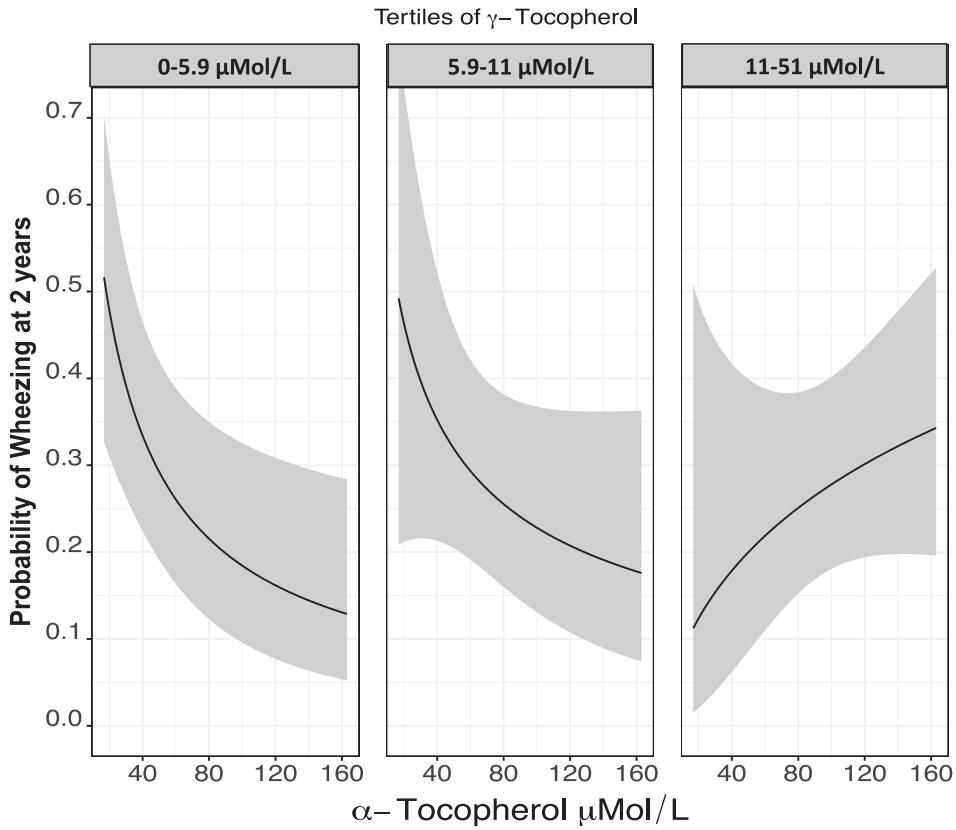

Figure 4. Effect modification of gamma-tocopherol concentrations on the association of alpha tocopherol with the outcome of 2-year wheezing assessed using multivariable logistic regression modeling. Model covariates included infant sex, race, birth weight, breastfeeding status, maternal smoking during pregnancy, maternal asthma, and insurance status. Alpha-tocopherol concentration is on the x-axis, and tertiles of gamma-tocopherol are represented in each panel. *P* interaction (likelihood ratio test) cross-product alpha- and gamma-tocopherol = .054.

individual isoform concentrations, may vary greatly.[45-48] Second, we demonstrate a protective effect on respiratory outcomes at normal physiologic levels of alpha-tocopherol, suggesting that high dose supplementation is not necessary. Because our observed associations were found within normal population ranges of tocopherols, any future intervention could likely be delivered primarily with dietary modification and avoid the known adverse effects of high dose supplementation.[48-50] Third, our data suggest that gamma-tocopherol, although protective at lower concentrations, is associated with increasing risk of wheeze with increasing concentrations.

One maternal vitamin E supplementation study on prevention of wheezing has been done, utilizing a daily 400 IU of RRR alpha-tocopherol co-administered with 1000 mg vitamin C during pregnancy, in a secondary analysis of data originally collected for the primary outcome of preeclampsia. This study, powered to detect a 30% decrease in wheezing, detected no difference for wheezing at age 2 years, aOR 0.97 (0.62-1.52), or for wheezing frequency of more than once per week at age 2 years, aOR 0.83 (0.26-2.59); there were nonsignificant reductions in use of respiratory medications, aOR 0.75 (0.50-1.13), $P = .068$.[51] Gamma-tocopherol effects were not accounted for in this study. Infants in the study were not typical of the usual population (multiple births, many admitted to neonatal special care.)

Strengths of our study include maternal tocopherol isoform ascertainment in over 600 INSPIRE participants, a diverse population-based study cohort, an analysis evaluating multiple confounders that would contribute to risk for developing wheezing over a 2-year period, and the consistency of our findings between children who wheezed both intermittently and recurrently. Despite the strengths, there are limitations that must be considered. We did not have maternal blood available during pregnancy to measure maternal vitamin E isoforms. However, we believe that our measurement following birth is a valid assessment of maternal status during pregnancy because of the slow changes in vitamin E equilibrium, as tocopherols are fat-soluble vitamins with predominantly extra-plasma stores. There was a trend with proximity to birth at enrollment and increased maternal tocopherol concentrations. This is likely because during pregnancy, maternal vitamin E plasma concentrations in women who are not taking vitamin E supplements gradually increase until delivery.[16,20] During the postpartum period, plasma concentrations gradually

decrease, resembling third trimester concentrations immediately after delivery, and second trimester concentrations by 6 weeks postpartum.[16,20] In sensitivity analyses that adjusted for the timing of postpartum sample collection in relation to infant age at enrollment, our results were not affected. Future studies will need to further explore the timing of isoform measurement in pregnancy and postpartum in relation to longitudinal risk of wheezing outcomes.[52] Another limitation is that we did not correct vitamin E isoform concentrations with lipid profiles, which are important for placing our measures in context of the overall dietary status of the mother. Because of the small volumes of blood from maternal finger stick, we were not able to correct for lipid profile and it is possible that a small number of our vitamin E isoform measurements have been altered by extremes of maternal lipid status,[53-55] though such adjustments may not be necessary.[56]

Our observations suggests that future preventive studies for asthma and allergic diseases should select formulations of vitamin E that quantify the content of both isoforms and contain predominantly alpha-tocopherol. These findings provide important new insights into the future design of interventions utilizing dietary modification for prevention of early life respiratory morbidity and recurrent wheezing, common and significant diseases of early childhood. Further study will be done as the INSPIRE cohort ages to evaluate the effects of maternal alpha- and gamma-tocopherol on the development of childhood asthma and allergy outcomes, and pathways through which they may exert a protective effect. ■

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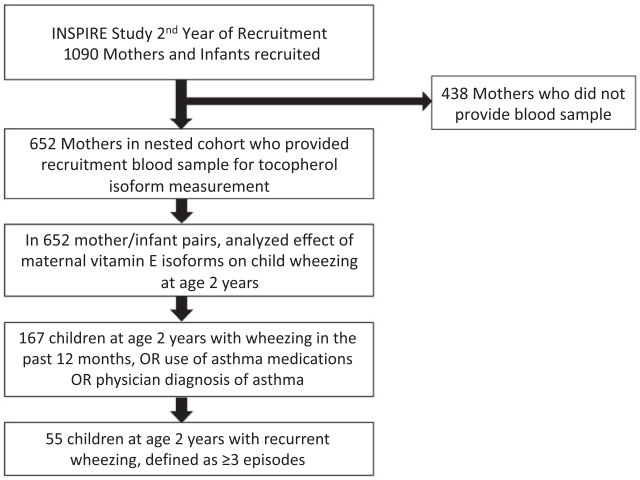

Figure 1. Flow diagram of this nested cohort study of maternal alpha- and gamma-tocopherol isoforms and the outcomes of 2-year wheezing and 2-year recurrent wheezing.

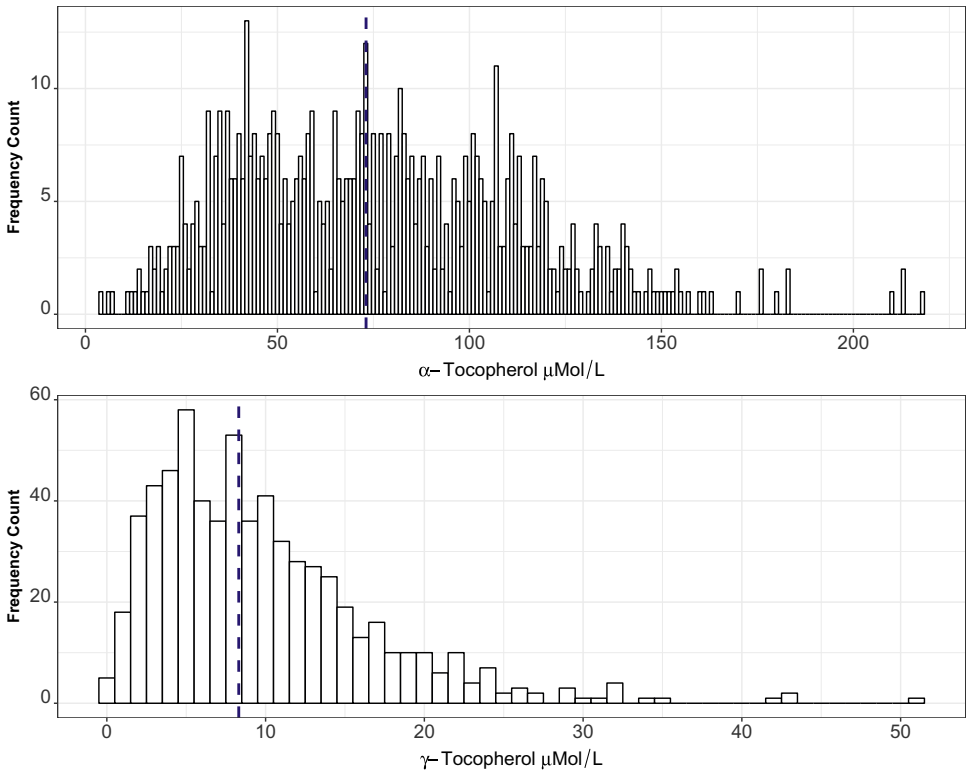

Figure 2. Distribution of maternal vitamin E isoforms in the study population (n = 652). Dashed vertical line denotes median value of each isoform for the population.

Table I. Study participants' demographic characteristics stratified by alpha tocopherol in tertiles (n = 652)

Variables	Alpha tocopherol 4.18-54.9 μmol/L n = 218	Alpha tocopherol 54.9-92.0 μmol/L n = 217	Alpha tocopherol 92.0-217.8 μmol/L n = 217	
Infants	**Median [IQR: 25th, 75th] or n (%)**	**Median [IQR: 25th, 75th] or n (%)**	**Median [IQR: 25th, 75th] or n (%)**	***P* value**
Gestational age (wk)	39 [39.0, 40.0]	39.0 [39.0,40.0]	39.0 [38.1,40.0]	.7
Infant age at enrollment (d)	63 [21, 97]	59 [18, 82]	24 [14, 65]	.001
Infant race or ethnicity				.035
Black	55 (25)	47 (22)	32 (15)	
White	123 (56)	134 (62)	143 (66)	
Hispanic	21 (10)	25 (12)	18 (8)	
Other	19 (9)	11 (5)	24 (11)	
Birth weight (g)	3405 [3126, 3717]	3405 [3121, 3717]	3518 [3206, 3859]	.028
Sex				.59
Female	109 (50)	98 (45)	102 (47)	
Male	109 (50)	119 (55)	115 (53)	
Parents				
Maternal age (ys)	25.0 [22.0, 30.0]	26.0 [23.0, 31.0]	28.0 [24.0, 32.0]	<.001
Postpregnancy BMI*	26.9 (24.0, 31.9)	28.1 (24.4, 33.1)	28.0 (24.2, 32.5)	.63
Mother ever smoked	82 (38)	85 (39)	82 (38)	.52
Smoked during pregnancy	46 (21)	41 (19)	28 (13)	.18
Prenatal vitamin use during pregnancy	202 (93)	197 (91)	210 (97)	.04
Breastfeeding at enrollment	101 (46)	108 (50)	122 (56)	.34
Maternal history of asthma	50 (23)	49 (23)	42 (19)	.61
Maternal history of allergies	59 (27)	59 (27)	53 (24)	.76
Paternal history of asthma	37 (17)	30 (14)	33 (15)	.86
Paternal history of allergies	58 (27)	58 (27)	50 (23)	.58

*BMI (kg/m^2) was available in n = 517 participants.

Table II. Study participants' demographic characteristics stratified by gamma tocopherol in tertiles (n = 652)

Variables	Gamma tocopherol 0-5.9 μmol/L n = 220	Gamma tocopherol 5.9-11.2 μmol/L n = 215	Gamma tocopherol 11.2-51.3 μmol/L n = 217	
Infants	**Median [IQR: 25th, 75th] or n (%)**	**Median [IQR: 25th, 75th] or n (%)**	**Median [IQR: 25th, 75th] or n (%)**	***P* value**
Gestational age (wk)	39.0 [39.0,40.0]	39.0 [39.0,40.0]	39.0 [38.0,40.0]	<.001
Infant age at enrollment (d)	54 [16,77]	48 [17,86]	44 [16,85]	.98
Infant race or ethnicity				.46
Black	48 (22)	46 (21)	40 (18)	
White	136 (62)	129 (60)	135 (62)	
Hispanic	24 (11)	17 (8)	23 (11)	
Other	12 (5)	29 (11)	19 (9)	
Birth weight (g)	3462 [3202,3775]	3405 [3107,3717]	3433 [3150, 3802]	.38
Sex				.88
Female	105 (48)	99 (46)	105 (48)	
Male	115 (52)	116 (54)	112 (52)	
Parents				
Maternal age (y)	27.0 [23.0, 32.0]	26.0 [23.0, 31.0]	26.0 [22.0, 30.0]	.094
Postpregnancy BMI*	26.4 [23.6, 30.6]	27.4 [24.5, 32.4]	29.2 [25.4, 34.4]	<.001
Mother ever smoked	64 (29)	85 (40)	91 (42)	.012
Smoked during pregnancy	26 (12)	51 (24)	38 (18)	.002
Prenatal vitamin use during pregnancy	207 (94)	202 (94)	200 (92)	.67
Breastfeeding at enrollment	129 (59)	106 (49)	96 (44)	.032
Maternal history of asthma	51 (23)	48 (22)	42 (19)	.6
Maternal history of allergies	68 (31)	61 (28)	42 (19)	.016
Paternal history of asthma	38 (17)	23 (11)	39 (18)	.14
Paternal history of allergies	64 (29)	57 (27)	45 (21)	.11

*BMI (kg/m^2) was available in n = 517 participants.

Table IV. Study participants' demographic characteristics, compared with the INSPIRE second year recruitment cohort

	Maternal vitamin E subcohort (n = 652)	INSPIRE second year cohort (n = 1090)
Infants	**Median [IQR: 25th, 75th] or n (%)**	**Median [IQR: 25th, 75th] or n (%)**
Gestational age (wk)	39 [39, 40]	39 [39, 40]
Infant age at enrollment (d)	50 [16, 80]	51 [16, 85]
Infant race or ethnicity		
Black	134 (21%)	204 (19%)
White	400 (61%)	702 (64%)
Hispanic	64 (10%)	97 (9%)
Other	54 (8%)	87 (8%)
Birth weight (g)	3433 [3150, 3774]	3405 [3121, 3717]
Sex		
Female	343 (53%)	576 (53%)
Male	309 (47%)	514 (47%)
Parents		
Maternal age at enrollment (y)	26 [23,31]	26 [22,31]
Post pregnancy BMI*	27.5 [24.2, 32.4]	27.4 [23.9, 32.4]
Mother ever smoked	240 (37%)	406 (37%)
Smoked during pregnancy	115 (18%)	211 (19%)
Prenatal vitamin use during pregnancy	609 (93%)	1015 (93%)
Breastfeeding at enrollment	331 (51%)	541 (50%)
Maternal history of asthma	141 (22%)	227 (21%)
Maternal history of allergies	171 (26%)	228 (21%)
Paternal history of asthma	100 (15%)	160 (15%)
Paternal history of allergies	166 (25%)	253 (23%)

*BMI (kg/m^2) was available in n = 517 of the vitamin E participants.