

Associations of α - and γ -tocopherol during early life with lung function in childhood



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Background: Tocopherol isoforms may regulate child lung growth and spirometric measures.

Objective: Our aim was to determine the extent to which plasma α -tocopherol (α -T) or γ -tocopherol (γ -T) isoform levels in early childhood or *in utero* are associated with childhood lung function.

Methods: We included 622 participants in the Project Viva cohort who had lung function at a mid-childhood visit (age 6–10 years). Maternal and child tocopherol isoform levels were measured by HPLC at the second trimester and 3 years of age, respectively. Multivariable linear regression models (adjusted for mid-childhood body mass index *z* scores, maternal education, smoking in pregnancy, and prenatal particulate matter with diameter of <2.5 micrometers (PM_{2.5}) particulate exposure) stratified by tertiles of child γ -T level were used to assess the association of α -T levels with FEV₁ and forced vital capacity (FVC) percent predicted. Similarly, models stratified by child α -T tertile evaluated associations of γ -T levels with lung function. We performed similar analyses with maternal second trimester tocopherol isoform levels.

Results: The median maternal second trimester α -T level was 63 μ M (interquartile range = 47–82). The median early-childhood level was 25 μ M (interquartile range = 20–33 μ M). In the lowest tertile of early-childhood γ -T, children with a higher α -T level (per 10 μ M) had a higher mid-childhood FEV₁ percent predicted (β = 3.09; 95% CI = 0.58–5.59 and a higher FVC percent predicted (β = 2.77; 95% CI = 0.47–5.06). This protective association of α -T was lost at higher γ -T levels. We did not see any consistent associations of second trimester levels of either α -T or γ -T with mid-childhood FEV₁ or FVC.

Conclusion: When γ -T levels were in the lowest tertile, a higher early-childhood α -T level was associated with better lung function at mid-childhood. Second trimester maternal plasma α -T concentration was 3-fold higher than in the adult nonpregnant female population. (J Allergy Clin Immunol 2020;146:1349–57.)

Key words: Tocopherol isoforms, lung function, child

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Asthma is a heterogeneous disease resulting from complex interactions of environmental and genetic factors that result in reduced lung function.¹ The World Health Organization reported that the prevalence of asthma from 1950 to the present has increased in countries regardless of whether they had high, intermediate, or low rates of asthma.^{2–4} The marked rise in rates of asthma over a few decades and the differences in rates among countries and in migrating populations suggest an important role for environmental factors, such as diet, in development of asthma. In Project Viva, food frequency questionnaire measures of prenatal vitamin E ingestion have been associated with increased measures of lung inflammation (fractional exhaled nitric oxide levels) in adolescence, but this has been without information on tocopherol isoforms.⁵ A better understanding of the tocopherol isoforms relevant for this association would be important, as reviewed by Strait et al.⁶ Indeed, an environmental change over the past 40 years that mirrors the rise in asthma prevalence has been an increase in dietary γ -tocopherol (γ -T) in both maternal diet and infant formulas. Infant formulas contain soybean oil, which is rich in γ -T,^{7–10} whereas breast milk contains about 10-fold higher levels of α -tocopherol (α -T) than γ -T.¹¹ A possible role of tocopherol isoforms on lung growth and inflammatory responses may be relevant to respiratory morbidity in this context.

γ -T is 1 of the 2 most abundant natural forms of tocopherols in diet and tissues, the other being dietary α -T.^{7–10} In preclinical studies in allergic asthma, these 2 isoforms have opposing effects on the allergic response, potentially via interactions with protein kinase C α (PKC α).^{12–14} In murine models, α -T blocks

Abbreviations used

α -T:	α -Tocopherol
γ -T:	γ -Tocopherol
IQR:	Interquartile range
PKC α :	Protein kinase C α
PM _{2.5} :	Particulate matter with diameter <0.25 micrometers
FVC:	Forced vital capacity

eosinophilic allergic responses whereas γ -T elevates eosinophilic allergic lung inflammation and airway responses.^{8,13-16} Also, γ -T ablates the anti-inflammatory benefit of α -T, even when the tissue concentration of γ -T is 10-fold lower than that of α -T, suggesting a potent effect of γ -T.^{13,17} In humans, α -T and γ -T isoforms may have opposing effects on both childhood wheeze¹⁸ and adult lung function and asthma. Specifically, in adults, α -T level is associated with better lung function and high γ -T level (>10 μ M) is associated with lower lung function by age 21 years,^{8,13,15,19} suggesting that early in life, α -T and γ -T may regulate lung function.¹⁹ Notably, high plasma γ -T level (>10 μ M) with low α -T level (12–15 μ M) also resulted in up to a 4-fold increase in the odds ratio for asthma in an 8-year study of adults in China.²⁰ However, prior studies have not assessed the role of tocopherol isoforms prenatally and in early childhood on later childhood lung function. This is important, as tocopherol isoforms in the diet are impactable, and plateau lung function has effects on life course events, including lung function trajectory, respiratory morbidity, and all-cause mortality.^{21,22}

The hypothesis that tocopherol isoforms may be relevant for lung inflammation is supported by our preclinical work with pregnant murine models. We demonstrated that α -T supplementation of mice with allergy that are pregnant or nursing decreases neonate allergic responsiveness²³ whereas γ -T supplementation increases it.²⁴ Moreover, murine studies indicate that when both of these tocopherol isoforms are administered together, γ -T ablates the benefit of α -T.^{13,17} Similar regulation of allergic inflammation by α -T *in utero* and after birth were noted in cross-foster studies.²³ This suggests that α -T and γ -T levels in combination with early-life events may be relevant to lung function in children. Indeed, α -T and γ -T may regulate early-life responses to lung events such as viral illnesses that effect both lung function and asthma.²⁵⁻³¹ Previous reports have not determined associations for maternal and child plasma α -T and γ -T levels with child spirometric measures.

We hypothesized that higher early-life or prenatal exposures to α -T would be associated with improved lung function. We secondarily hypothesized that these associations would be abrogated at higher levels of γ -T. To examine these hypotheses, we measured plasma tocopherol isoform levels in early childhood and in maternal second trimester plasma (a time that is relevant to the development of adaptive immunity in the fetus) and determined their associations with childhood lung function in the Project Viva cohort.

METHODS

Cohort

Project Viva is a US cohort study of pregnant women and their offspring registered at [ClinicalTrials.gov](https://clinicaltrials.gov) as NCT02820402. The Viva cohort enrolled pregnant women in the first trimester from Atrius Harvard Vanguard Medical

Associates in eastern Massachusetts between 1999 and 2002, with 2128 live births and follow-up of about 1600 mother-child dyads to the present time. Exclusion criteria included multiple gestations, inability to answer questions in English, gestational age at least 22 weeks at recruitment, and plans to move away before delivery. Additionally, for this analysis, we excluded individuals who had a gestational age less than 34 weeks.³² The institutional review board of Harvard Pilgrim Health Care approved this study protocol, and mothers provided written informed consent at recruitment and follow-up.

For this analysis, we focused on a subset of subjects who had either second trimester maternal plasma or early-childhood (age 3 years) plasma and had a mid-childhood visit (at age 6–10 years) with spirometry testing. Additional details are described elsewhere.³³ We analyzed plasma samples for tocopherol isoforms from 1117 participants, of whom 622 also had data on mid-childhood spirometry.

Tocopherol measurements

Concentrations of tocopherol isoforms were determined by HPLC with an electrochemical detector in plasma from mothers in the second trimester and child plasma. Briefly, 7 μ L of 0.2 N HCl was added to each plasma sample (50 μ L) on ice and vortexed. Then the following substances were added in consecutive order: the internal standard tocol (20 μ L of 0.05 mg/mL tocol [Abcam, Cambridge, Mass]), 2 μ L of 10% ascorbic acid in water, 0.5 mL of 100% ethanol (HPLC-grade), 0.5 mL of water, and 1 mL of hexane with 0.1% wt/vol butylated hydroxytoluene to prevent oxidation and increase recovery of tocopherol. The samples were vortexed and then centrifuged for 3.5 minutes at 9000 g at 4°C and the hexane layers were collected. The samples were extracted a second time with addition of hexane/butylated hydroxytoluene. The hexane layers were combined, dried under nitrogen, and stored at –20°C. The samples were reconstituted in methanol and the tocopherols were separated by using a reverse-phase C₁₈ HPLC column (catalog No. WAT085711 [Waters Co, Milford, Mass]) and HPLC chromatograph (Waters Co) with 99% methanol and 1% water as a mobile phase with detection with an electrochemical detector (potential 0.7 V) (Waters Co). Tocol standards that were equivalent to 100% of tocol added to the samples were measured after every 7 samples. Standard curves containing tocol, α -T, γ -T, β -tocopherol, and δ -tocopherol (25- μ L injection volume of 15, 10, 5, 2.5, 1.25, 0.6, 0.3, and 0.15 μ g of standards/mL) were done every 3 days of the analysis, with a lower limit of detection of less than 0.003 μ g of tocopherol. The levels of β -tocopherol and δ -tocopherol were near or below the detection limit in all the plasma samples. Concentrations were calculated by using the area under the curve of the HPLC chromatograms, the standard curves, and tocol percent recovery from extractions. All samples were analyzed within the linear standard curve, and additionally, maternal samples with high levels of α -T were periodically reextracted and analyzed to confirm the accuracy of concentration in previous samples. Our coefficient of variation for tocopherol analysis of human plasma is 6% to 8%, consistent with reports of less than 10%.¹⁹

Pulmonary function measurements

Lung function measurements, including FEV₁, FVC, and forced expiratory flow at 25% to 75% of FVC, were obtained by using the EasyOne Spirometer (NDD Medical Technologies, Andover, Mass). Spirometry measurements met the American Thoracic Society criteria for acceptability and reproducibility: for inclusion, each child had to produce at least 3 acceptable spirometry, 2 of which had to have been reproducible.³⁴ Mothers were instructed to hold any child inhaler medications on the day of spirometry testing.³⁵ Results were expressed as percent predicted by using the Global Lung Function Initiative equations.³⁶

Statistical analysis

Descriptive statistics summarized all variables, including α -T and γ -T levels and potential confounders. Demographic and clinical variables were compared between the subgroup included in analyses and the excluded subgroup via chi-square tests. All analyses were specified *a priori* on the basis of mechanisms defined in preclinical studies.^{8,13-17,23,24} Linear regression models, stratified by tertiles of child γ -T, were used to assess the association

of α -T levels (in 10- μ M increments) with FEV₁ and FVC percent predicted at 7 years of age. Similarly, models stratified by child α -T tertile considered the association of γ -T levels (in 10- μ M increments) with FEV₁ and FVC percent predicted at the 7-year visit (mean age, 7.9 years; range, 6.6-10.6; SD = 0.8 year). Analyses were replicated in a similar fashion for maternal second trimester tocopherol levels.

Unadjusted linear regression models with the outcome FEV₁ percent predicted were also used to evaluate potential confounders, including gestational age, maternal age at enrollment, maternal education, household income, smoking during pregnancy, exposure to passive smoke postnatally, child body mass index *z* scores at the mid-childhood (7-year) visit, and pollution variables (including distance to major roadways, traffic density, prenatal exposure to particulate matter with a diameter <2.5 micrometers [PM_{2.5}], and prenatal BC). Sex, race, height, and age of the child were not considered because percent predicted outcomes already adjust for these variables. There were a number of covariates that were retained in the model as potential confounders (associated with the outcome with an alpha level of $P \leq .2$), including maternal education level, smoking during pregnancy, and prenatal PM_{2.5} air pollutant exposure. Maternal education was dichotomized as less than 4 years of college versus 4 or more years of college. Any self-report of maternal smoking on standardized questionnaires administered at study visits during pregnancy and before delivery was coded as "maternal prepregnancy smoking," with never-smokers or former smokers classified as not smoking during pregnancy. Prenatal PM_{2.5} air pollutant exposure referred to period averages from measures taken from each trimester on the basis of temporally and spatially resolved, aerosolized optical depth data.³⁷ For consistency, these variables were also included in multivariable models with the FVC percent predicted outcome. All models with child 3-year tocopherols were also adjusted for some hemolysis, which occurred in 22% of the samples because lipids are released from cell membranes during hemolysis. Similar models were also fit within the subgroup of subjects or a history of wheezing at mid-childhood, additionally controlling for controller medication use as a confounder to further explore the relationship between tocopherol levels and lung function. We classified subjects as taking a controller medication in the last year if they had used any of the following: inhaled steroid, prednisone, nedocromil, montelukast, theophylline, and an inhaled corticosteroid and/or long-acting β -agonist.

Exploratory analyses considered *t* tests or Wilcoxon rank sum tests, as appropriate, to compare FEV₁ and FVC percent predicted between 2 subgroups of the cohort, defined by levels of α -T and γ -T at age 3 years. Specifically, the high-alpha and low-gamma subgroup was defined as subjects with an α -T level greater than 40 μ M and a γ -T level not exceeding 7 μ M. The high-gamma and low-alpha subgroup was defined as subjects with an α -T level not exceeding 40 μ M and a γ -T level greater than 7 μ M. These values were selected on the basis of the mean and 1.5 SDs within this cohort as in the CARDIA study.¹⁹ Analyses were replicated with subgroups defined by maternal tocopherol levels, with the high-alpha and low-gamma subgroup defined as subjects with an α -T level greater than 100 μ M and a γ -T level not exceeding 10 μ M; the low-alpha and high-gamma subgroup was defined as subjects with an α -T level not exceeding 100 μ M and a γ -T level greater than 10 μ M.

Additional exploratory analyses compared the distributions of α -T and γ -T by prenatal vitamin intake by using Wilcoxon rank sum tests. Unless otherwise specified, analyses assumed a 2-sided type 1 error rate of 0.05 and no adjustments for multiple tests.

RESULTS

Sample characteristics

Of the 1117 subjects with second trimester or early-childhood exposure data, we included 622 mother-infant dyads with data for lung function in mid-childhood (age 6-10 years). Compared with those in the Project Viva cohort who were excluded from the analysis (1506), the sample of 622 dyads were not significantly different with respect to sex of the infants ($P = .2$), race/ethnicity ($P = .1$), or proportion whose mothers had asthma or eczema as measures of maternal atopy ($P = .2$). The mean difference in

TABLE I. Demographics of the included Project Viva subjects

Variable	n (%)	Median (IQR)
Median maternal age at enrollment, y	622 (100)	32.5 (29.3-35.9)
Gestational age (wk)	622 (100)	39.7 (38.9-40.6)
Total IgE level, kU/L (early childhood to age 3 y)	400 (64.3)	25.7 (9.7-73.4)
Sex		
Male	307 (49.4)	—
Female	315 (50.6)	
Mid-childhood BMI <i>z</i> score		
Male		16.29 (15.30-17.77)
Female		16.58 (15.23-18.33)
Race		
White	410 (65.9)	—
Black	101 (16.2)	
Other	111 (17.9)	
Maternal education		
<4 y of college	176 (28.3)	—
≥4 y of college	445 (71.5)	
Missing	1 (0.2)	
Maternal prenatal vitamin use	477 (96)	
Tocopherols		
α -T level in 3-year-old child (μ M)	397 (63.8)	25.5 (20.1-33.3)
γ -T level in 3-year-old child (μ M)	397 (63.8)	2.6 (1.3-4.0)
α -T level in second trimester (μ M)	549 (88.3)	62.6 (46.7-82.0)
γ -T level in second trimester (μ M)	549 (88.3)	4.5 (3.0-6.5)
Children ever diagnosed with asthma or wheeze by questionnaire	207 (33.4)	—
Frequency of controller medication in children with asthma or wheeze at age 7 y		
Inhaled steroid use	84 (41.2)	
Leukotriene antagonist use	17 (8.3)	
Nedocromil	2 (1)	
Inhaled corticosteroid/long-acting β -agonist use	2 (1)	
Wheezing phenotype		
Never-wheezing children	299 (48.5)	
Transient wheeze	152 (24.7)	
Persistent wheeze	120 (19.5)	
Late-onset wheeze	45 (7.3)	
Lung function outcomes in mid-childhood (age 6-10 y)		
FEV ₁ percent predicted (age 6-10 y)	622 (100)	96.3 (86.7-104.3)
FVC percent predicted	622 (100)	102.5 (95.1-110.6)
FEV ₁ /FVC percent predicted	622 (100)	93.7 (88.7-97.8)

BMI, Body mass index.

maternal age at enrollment was less than 1 year. The included sample was, however, more likely to have higher education (≥ 4 years of college) compared with those excluded.

In Table I, we display the demographic characteristics of the analysis sample. The median (IQR) age of the mothers was 32.5 (29.3-35.9) years, with 51% of infants being female. The median FEV₁ was 96.3 (IQR = 86.7-104.3) percent predicted, and the FVC was 102.5 (IQR = 95.1-110.6) percent predicted. The median total IgE level was 25.7 kU/L (IQR = 9.7-73.4 kU/L). The proportion of children who were ever diagnosed with asthma or wheezing/reactive airways disease was 33%. We also classified subjects as having never wheezed (48.5%), having transient wheeze (24.7% had wheezing at any time up to 6 years of age and not beyond that), having persistent wheeze (19.5% had wheezing up to age 5 and continuing to have wheezing at any

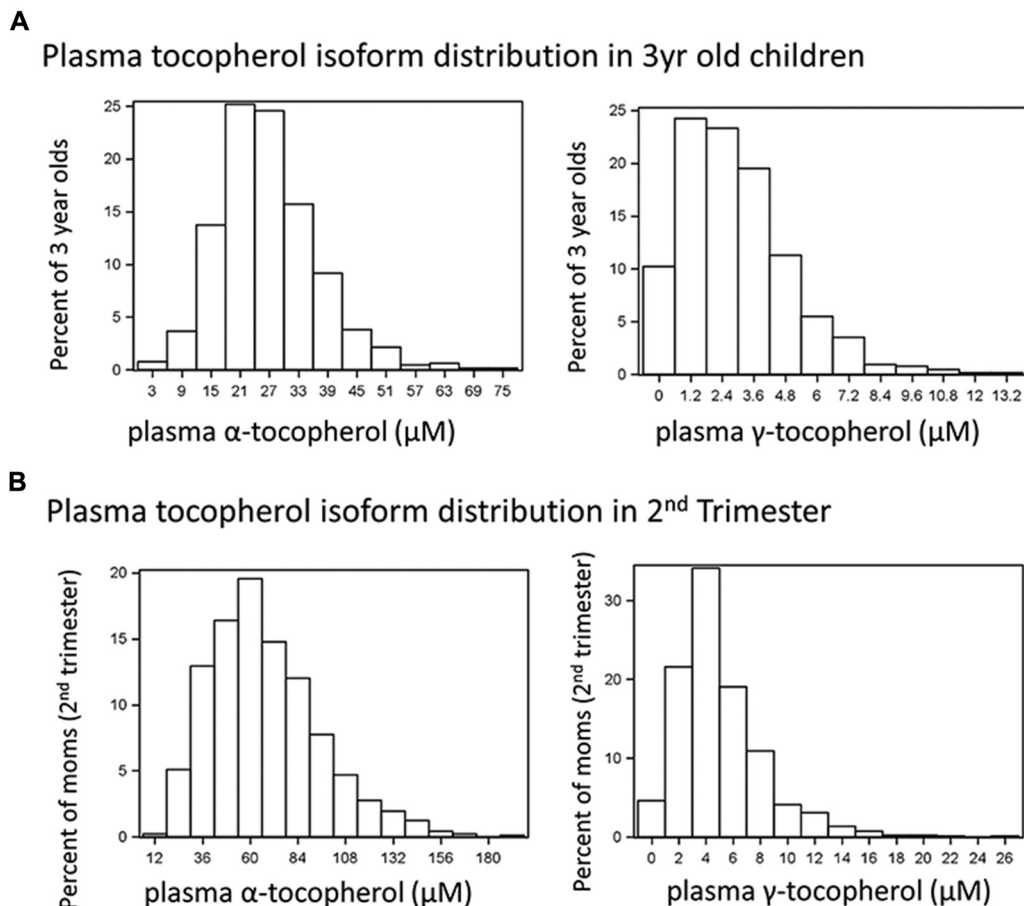


FIG 1. Tocopherol isoform distributions in children and in mothers. The x-axes for the maternal and child levels differ because of the dramatically greater maternal tocopherol levels.

of the visits at 7, 8, or 9 years of age), and having late-onset wheeze (7.3% had no wheezing up to age 5 years, but onset of wheezing at any of the visits at 6, 7, 8, or 9 years of age). There were no clinically meaningful differences in percentage of subjects with asthma and wheeze by tertile of each tocopherol isoform (see [Table E1](#) in this article's Online Repository at www.jacionline.org). We also present the levels of each tocopherol isoform between those subjects with asthma (or a history of wheezing) who were taking a controller medication versus those who were not taking such medications (see [Table E2](#) in this article's Online Repository at www.jacionline.org).

Distribution of tocopherol measures

[Fig 1, A](#) displays the distribution of tocopherol isoforms in the children at age 3 years from the entire study population with this information ($n = 656$ with tocopherol levels at age 3 years and $n = 982$ with second trimester tocopherol levels). The median child plasma α -T level was $25.48 \mu\text{M}$ (range 2.95 - $73.16 \mu\text{M}$) and the median child plasma γ -T level was $2.67 \mu\text{M}$ (range 0.00 - $13.52 \mu\text{M}$). The α -T levels were higher than the prior reference ranges in children (11.9 - $30 \mu\text{M}$)³⁸ but similar to the average adult α -T levels.^{19,39} [Fig 1, B](#) displays the distribution for tocopherol isoforms in the mothers measured in the second trimester,

revealing markedly higher levels of α -T (median, $63.13 \mu\text{M}$ [range, 14.84 - $192.66 \mu\text{M}$]) than the average α -T level in nonpregnant adults¹⁹ and the average levels of γ -T in pregnant women (median, $4.40 \mu\text{M}$ [range, 0.00 - $26.39 \mu\text{M}$]). In all, 89% of mothers took prenatal vitamins and 96% used prenatal vitamins or multivitamins, but there were no statistically significant differences in α -T levels between those who reported prenatal vitamin use and those who did not (see [Table E3](#) in this article's Online Repository at www.jacionline.org). We elected to categorize these measurements in tertiles to determine the associations of the opposing isoform of tocopherol in different strata of the variable, as illustrated in [Table II](#).

Association of tocopherol isoform levels with lung function in children at the mid-childhood visit (age 6-10)

Given the opposing associations of γ -T and α -T on adult lung function¹⁹ and the preclinical data, which showed that effects of either isoform were evident only in subjects with low levels of the other,^{13,23,24} we analyzed associations of opposing effects of tocopherol isoforms. The association of each isoform was analyzed by strata based on tertiles of the opposing tocopherol.

TABLE II. Plasma levels and tertiles of tocopherol isoforms in mothers in the second trimester of pregnancy and in children at 3 years of age

Variable	α -T in mothers in second trimester	γ -T in mothers in second trimester	α -T in children at age 3 y	γ -T in children at age 3 y
Median tocopherol isoform level, μ M (IQR)	63.13 (47.49-83.50)	4.40 (2.93-6.38)	25.48 (19.78-32.41)	2.67 (1.43-4.02)
Tertiles				
First	≤ 53.326	≤ 3.428	≤ 21.503	≤ 1.733
Second	53.329-75.945	3.429-5.471	21.504-29.520	1.734-3.538
Third	≥ 75.946	≥ 5.472	≥ 29.521	≥ 3.539

TABLE III. Association of tocopherol isoform levels (in 10- μ M increments) within tertile strata of the opposing tocopherol isoform with lung function at the mid-childhood visit (at age 6-10 years)

Predictor (per 10- μ M increment)	Tertile of opposing isoform	n	Change in % predicted FEV ₁ per 10- μ M increment			Change in % predicted FVC per 10- μ M increment		
			β	95% CI	P value	β	95% CI	P value
γ -T at age 3y	α -T 3y (1)	82	17.40	-6.46 to 41.26	.15	11.11	-9.87 to 32.09	.30
	α -T 3y (2)	86	-1.90	-14.2 to 10.41	.76	-6.41	-17.80 to 4.94	.26
	α -T 3y (3)	103	-5.71	-16.90 to 5.50	.31	-5.13	-15.60 to 5.37	.33
α -T at age 3 y	γ -T 3y (1)	103	3.13	0.63-5.84	.01	2.85	0.58-5.12	.01
	γ -T 3y (2)	80	-0.19	-3.58 to 2.3.20	.91	-1.39	-4.47 to 1.69	.37
	γ -T 3y (3)	88	-0.89	-4.71 to 2.93	.64	0.59	-3.24 to 4.42	.86
γ -T in Tri 2	α -T Tri 2 (1)	107	4.44	-9.41 to 18.28	.53	1.68	-11.1 to 14.45	.79
	α -T Tri 2 (2)	135	-6.49	-14.50 to 1.47	.11	-10.20	-17.1 to -3.30	.004
	α -T Tri 2 (3)	116	-0.71	-7.96 to 6.54	.85	-4.09	-11.3 to 3.06	.26
α -T in Tri 2	γ -T Tri 2 (1)	111	0.25	-0.87 to 1.37	.66	0.25	-0.73 to 1.22	.62
	γ -T Tri 2 (2)	131	0.42	-0.79 to 1.63	.50	0.45	-0.68 to 1.58	.43
	γ -T Tri 2 (3)	116	0.79	-0.04 to 1.62	.06	0.54	-0.32 to 1.40	.22

BMI, Body mass index; Tri 2, second trimester.

Data are adjusted for education level, smoking during pregnancy, and prenatal exposure to particulate matter (PM_{2.5}), as well as child BMI z-scores based on CDC 2000 reference data at age 7 years; 3-year exposure models are also adjusted for hemolysis.

In Table III, we display the associations of both maternal second trimester and child tocopherol at age 3 years with percent predicted FEV₁ and FVC at the mid-childhood visit (at age 6-10 years), adjusted for maternal education level, smoking in pregnancy, child body mass index z score in mid-childhood (7-year visit), prenatal PM_{2.5}, and presence of some hemolysis in some of the 3-year samples. In the lowest tertile of γ -T levels, children with higher α -T levels (per 10 μ M) had a higher expected FEV₁ percent predicted ($\beta = 3.13$; $P = .01$) and a higher FVC percent predicted ($\beta = 2.85$; $P = .01$). We evaluated the same associations with maternal levels of tocopherol isoforms. Notably, with the very high levels of α -T in maternal plasma, we did not see any consistent associations for child year 7 FEV₁ and FVC with second trimester levels of either α -T or γ -T. A finding for maternal tocopherols was that for each 10- μ M increment in γ -T level, individuals in the middle tertile of second trimester α -T had a decrement in percent predicted FVC only ($\beta = -10.20$; $P = .004$).

To further examine the association of γ -T with lung function in children with a low α -T level (in quadrants 1 and 4 of Fig 2, A-C), we carried out secondary analyses in children and mothers. In these unadjusted analyses shown in Fig 2, C, we noted that those children with high α -T levels (>40) and low γ -T (≤ 7) at age 3 years had greater FEV₁ % predicted values at the mid-childhood visit (at age 6-10 years) than did those who had high levels of γ -T (>7) and low levels of α -T (≤ 40) (97.74 ± 11.75 percent predicted versus 88.02 ± 11.75 percent predicted, respectively), which is consistent with opposing

functions of tocopherol isoforms on lung spirometry in young children. This association remained present when limited to children with asthma even while controlling for use of a controller medication (for each 10-IU increase in α -T, FEV₁ increased by 4.43% [95% = CI 0.39-8.46] and FVC increased by 4.36% [95% CI = 0.26-8.47]). When we applied this approach to maternal second trimester plasma levels, which included very high levels of α -T, we did not find a significant difference in lung spirometry results at the mid-childhood visit (age 6-10 years).

DISCUSSION

We sought to evaluate the association of early-life plasma levels of tocopherol isoforms with mid-childhood lung function, especially because there are opposing associations of tocopherol isoforms with plateau lung function in adults by age 21.¹⁹ This is the first population-based study that evaluated the association of plasma levels of tocopherol isoforms in young children with lung function. We found that a higher α -T level in 3-year-olds was associated with better lung function at the age of 7 years, when γ -T levels were in the lowest tertile. This protective effect of α T was lost at higher γ -T levels. Interestingly, the mean second trimester mother α -T concentrations in plasma was 3-fold higher than that reported for nonpregnant female adults, whereas γ -T concentrations in the second trimester were similar to the γ -T levels of nonpregnant adult.^{19,39} Moreover, the 75th percentile

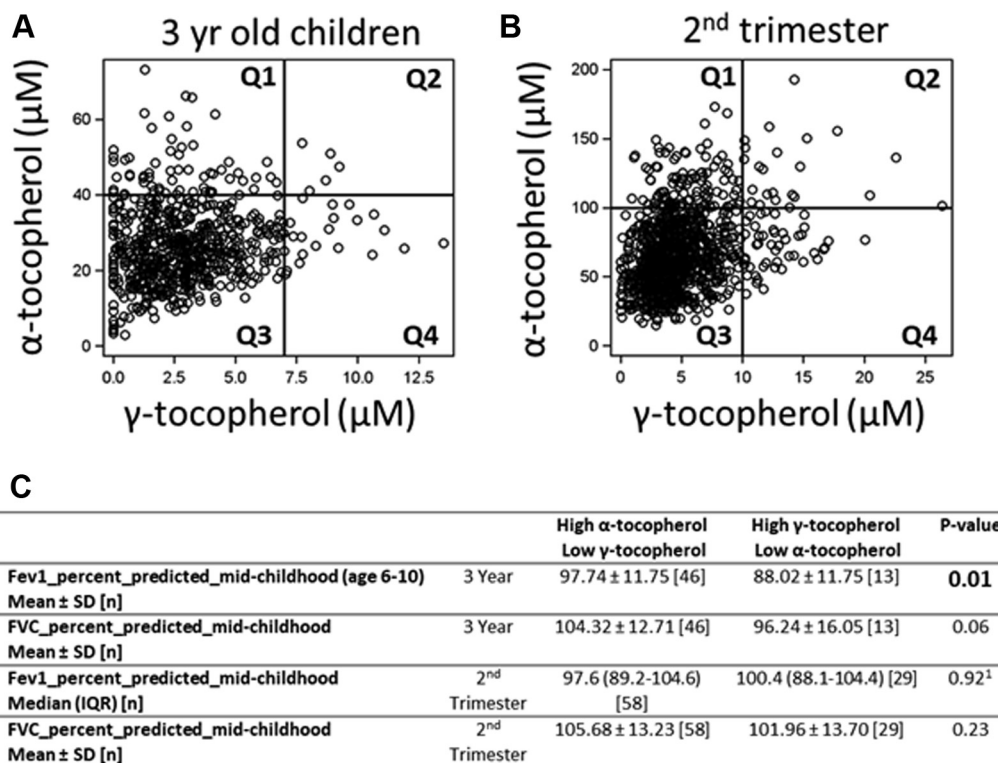


FIG 2. Lung function between children with predominantly high α-T level (and low γ-T level) and children with high γ-T level (and low α-T level). Distribution of α-T and γ-T among 3-year-olds (A) and second trimester mothers (B). The 4 quadrants are labeled Q1, Q2, Q3, and Q4, respectively. C, Comparison of lung function for subjects in quadrants Q1 versus Q4 of (A) and (B). ¹The nonparametric Wilcoxon rank sum test was used for P value; all other P values were obtained from a t test. The data for FEV₁ were not normally distributed in the sample with second trimester tocopherol data.

of second trimester α-T level was about 5 times higher than the reported average α-T levels in nonpregnant women in the United States.⁴⁰ This high level of α-T in second trimester plasma is similar to the pregnancy levels in Australia in the second trimester, with a high level of α-T (43 μM) and normal levels of γ-T (1.4 μM) reported for adults in Australia.⁴¹ Plasma tocopherol levels in pregnancy have been measured previously,⁴²⁻⁴⁴ but their associations with childhood lung function have not been reported before. Of note, this elevation in second trimester α-T level did not seem to differ by reported prenatal vitamin use. It is unclear whether this is due to inaccurate reporting of prenatal vitamin use or there may be other factors such as upregulation of α-T transfer protein and active transport, which may mediate these high α-T levels. Also, changes in α-T metabolism may occur during pregnancy in humans.⁴⁵ In the presence of these high second trimester α-T plasma levels, the maternal tocopherol concentrations were not associated with lung function in a 7-year-old child.

Our prior epidemiologic findings in adults suggest that α-T and γ-T tocopherol isoforms may have opposing effects on lung function. In a normative aging study, γ-T was associated with lower lung function.⁴⁶ In a prospective 20-year cohort (CARDIA), we demonstrated that a 5-fold higher plasma α-T level is associated with better lung spirometry and that a 5-fold higher plasma γ-T level is associated with lower lung spirometry results (a 10% to 17% decrease in FEV₁) in adults by age 21,^{8,13,15,19} suggesting that early in life, α-T and γ-T may regulate lung

development, potentially by mediating responses to environmental exposures.¹⁹ This hypothesis that tocopherol isoforms may be relevant for both lung growth and asthma development is supported by our preclinical work with pregnant murine models. We have demonstrated that α-T supplementation of pregnant mice with allergy decreases²³ neonate responsiveness to allergen, whereas γ-T supplementation of pregnant mice increases neonate responsiveness to allergen.²⁴ In our mechanistic mouse studies with cross-fostering of pups at birth, we demonstrated a contribution to regulation of allergic inflammation by α-T *in utero* and after birth.²³ This suggests that α-T and γ-T levels in combination with early-life events may be relevant to both asthma and lung development. Indeed, early-life severe lower respiratory viral illnesses are associated with asthma and worse lung function, with potential fixed obstruction in some subjects in later childhood.^{47,48} Furthermore, prior studies from the Viva cohort have suggested that vitamin E (tocopherol isoforms) may regulate early-life responses and wheezing to lung events such as viral illness, which would affect both FVC and asthma.²⁵⁻³¹ The interaction of tocopherol levels with specific viral illness could not be addressed directly in the current study in the absence of virology. Furthermore, self-report of viral illness used in this study would be poorly sensitive, with only 5% recalling a clinical diagnosis of bronchiolitis. With respect to rhinovirus, random sampling over a year in 1 cohort of children revealed that 50% of the children had detectable virus, with 72% to 99% of the samples proving to be rhinovirus.⁴⁹ Similarly,

19% of children under the age of 5 years have had RSV, and the rate increases to 95% by the time the children are 5 years of age.^{50,51} Further prospective studies with virology may help delineate the effects of any interaction of specific viral insults with tocopherol levels on later life lung function. Although we recognize that FEV₁ is typically more proportionally affected, pediatric asthma can have both lowered FEV₁ and FVC.²⁵⁻³¹ Along these lines, we also analyzed the outcome of childhood asthma (data not shown) but found no association with tocopherol isoforms. We assume that this is due to power considerations (insufficient numbers in subgroups with low levels of the opposing tocopherol) for this dichotomous and multifactorial outcome. Our findings are also important in that if tocopherols are important in early lung development, they may be modifiable risk factors for impaired lung function growth and early-life risks for later lung disease in adulthood.⁵²

The opposing effects of γ -T and α -T isoforms, the 2 most abundant natural forms of tocopherols in diet and tissues,⁷⁻¹⁰ on lung function is biologically plausible. There are a number of mechanisms previously postulated for the effects of tocopherols on the developing immune system, including decreased cord blood mononuclear cell responses to antigen stimulation,⁵³ decreased IL-4 production in T cells,⁵⁴ and decreased sensitivity of DCs to proinflammatory cytokines.⁵⁵ These all could have relevance to both response to viral insult and the development of asthma. Indeed, in our preclinical mechanistic studies of signals for eosinophil recruitment in allergic asthma, we demonstrated that γ -T is an agonist and α -T is an antagonist of recombinant PKC α and that α -T and γ -T bind to a regulatory domain of PKC α ,¹²⁻¹⁴ a molecule important in airway viral infection,⁵⁶⁻⁵⁸ T-cell responses,⁵⁹ and eosinophil recruitment to tissues.^{8,13-16} *In vivo*, we demonstrated that a 5-fold increase in α -T blocks eosinophilic allergic responses (a 65% decrease) in adult mice whereas a 5-fold increase in γ -T elevates eosinophilic allergic lung inflammation (175%) and airway responses in adult mice.^{8,13-16} γ -T potentially elevates allergic inflammation in preclinical models. Tissue levels of γ -T are 10-fold lower than tissue levels of α -T because α -T is preferentially transferred to liver lipoproteins by α -T transfer protein.⁶⁰⁻⁶⁴ At 10-fold lower tissue levels, in adult mice, γ -T elevates allergic responses to ovalbumin^{13,17} and house dust mite extract²⁰ and potentially ablates the anti-inflammatory benefit of α -T during allergic responses.^{13,17} γ -T amplification of allergic lung inflammation in adult mice is partially reversible by switching to elevated α -T level before allergen challenge.¹⁵ These findings of opposing effects of tocopherols in disease and lung development with effects of α -T blocked at high levels of γ -T may have relevance to prior conflicting results from vitamin E clinical trials on the modulation of established disease.³⁹ Specifically, in the US population no effect of tocopherol on asthma severity was seen, but the mean serum γ -T concentration in the US population was 2- to 6-fold higher than in the French and Italian populations, where there were clear effects.⁶⁵⁻⁶⁸ Finally, because of sample size, we were not able to examine interactions between markers of TH2 predisposition such as eosinophilia and predominant γ -T levels (high levels of γ -T and low levels α -T) on the outcome of lung function.

The 9% difference in percent predicted FEV₁ at the mid-childhood visit (age 6-10 years) comparing the children at the opposite spectrum of the tocopherol isoform distributions (high α -T plus low γ -T compared with low α -T plus high γ -T) at age

3 is clinically relevant. Even in subjects with a diagnosis of asthma or wheezing at any point, this level of difference in FEV₁ is larger than the amount of difference seen with allergen sensitization noted by a large longitudinal study.⁶⁹ The lower lung function in these Viva participants is similar to the 5% to 11% reduction in lung function reported for other environmental factors. It is reported that responders to cotton have a 5% to 8% decrease in FEV₁ and that this decrease is associated with dyspnea, chest tightness, chronic bronchitis, and chronic cough.^{70,71} It is also reported that responders to particulate matter have a 2% to 6% decrease in FEV₁,⁷² responders to cold or exercise have a 5 to 11% decrease in FEV₁,⁷³ and responders to house dust mite or dog/cat dander have a 2% to 8% decrease in FEV₁.⁷⁴ These subsets represent the extremes of the spectrum, where there is minimal competition of the opposing tocopherol isoform. However, even while looking at the children with the lowest tertile of γ -T, the difference in α -T across the IQR for the population (13 μ M) is associated with a 4% difference in percent predicted FEV₁. To put this difference in perspective, this is the same level of difference in FEV₁ percent predicted that is seen across the spectrum of pediatric asthma as reported by Bacharier et al.⁷⁵

Interestingly, we did not see associations of child lung function with *in utero* tocopherol isoform exposures. The levels of maternal α -T concentrations in plasma were at least 3-fold higher than those reported for nonpregnant female adults, whereas the γ -T concentrations in the second trimester were similar to nonpregnant levels. This suggests that the high levels of second trimester α -T in these mothers, which were above the average for nonpregnant women, may be at maximum for child lung function outcomes. This is the first report that examines the association of childhood lung function with plasma levels of tocopherols in pregnancy. The high α -T during pregnancy likely reflects a combination of the overall increase in lipid transport during pregnancy, potential increases in the liver α -T transfer protein during pregnancy, potential changes in α -T metabolism, and increase in intake of α -T in prenatal vitamin supplements.

The study has potential limitations. First, we do not have serial measurements of tocopherol levels in childhood between the age of 3 years and the mid-childhood visit (age 6-10 years). It would be important to determine whether there is a critical period in which lung growth and development are more affected. Additionally, we made no formal adjustment for multiple testing because the analyses were selected on the basis of preclinical mechanistic outcomes; however, lung function outcomes are correlated and do not represent independent outcomes. Furthermore, the associations tested were established *a priori* at the onset of the project, with lung function as the primary analysis and all others as secondary. These novel findings in children are consistent with the suggestion that in adults, higher levels of α -T in the context of lower levels of γ -T are associated with higher plateau lung function (and presumably lung development) in our studies in the CARDIA study¹⁹ and are consistent with the differential effects of these tocopherol isoforms in animal models.^{8,13-16,23} Although we do not see the same degree of effect of γ -T at age 3 years that we saw in our CARDIA article,¹⁹ these data in the Project Viva cohort demonstrate the novel finding that in early childhood, children with high levels of the α -T isoform and low levels of the γ -T isoform have higher lung function than do those children with high levels of the γ -T isoform and low levels of the α -T isoform at mid-childhood. This finding is more direct evidence than are cross-sectional associations with plateau lung

function. Additional studies in larger numbers of subjects are needed to further delineate any opposing effects of α -T and γ -T in lung development. Finally, further mechanistic *ex vivo* studies in humans will help to examine mechanism.

In summary, our findings and the previous preclinical studies suggest that early-childhood consumption of tocopherol isoforms could influence childhood lung function. Although we did not see similar associations with *in utero* exposure to tocopherols and lung function, it is possible that this is due to a potential maximal effect of the levels of α -T in pregnancy in the Project Viva cohort. However, on the basis of our findings, lung function in childhood may be modifiable by α -T and γ -T levels early in childhood regardless of the prenatal vitamins. Further studies in prospective trials are needed to determine whether changes to tocopherol isoforms in formula and diet would improve lung function trajectory in childhood and into adult life.

Clinical implications: α -T and γ -T isoforms are potentially modifiable exposures that have differential associations with lung function in later childhood.

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TABLE E1. Distribution of asthma and wheezing by tertile of each isoform

Isoform studied in children and pregnant mothers	Tertile of opposing tocopherol isoform	No asthma and no wheeze by age 7 y (row %)	Asthma or wheeze by age 7 y (row %)
Isoform level in children at the age of 3 y			
α -T	1	72 (55.8)	57 (44.2)
	2	93 (73.2)	34 (26.8)
	3	85 (61.2)	54 (8.9)
γ -T	1	93 (64.6)	51 (35.4)
	2	73 (60.8)	47 (39.2)
	3	84 (64.1)	47 (35.9)
Isoform level in mothers in the second trimester of pregnancy			
α -T	1	121 (66.1)	62 (33.9)
	2	130 (68.4)	60 (31.6)
	3	121 (69.5)	53 (30.5)
γ -T	1	118 (66.7)	59 (33.3)
	2	131 (69.7)	57 (30.3)
	3	123 (67.6)	59 (32.4)

This table is based on parent reports of a physician diagnosis of asthma at any point or a report of wheezing at any point on the questionnaires up to the mid-childhood (year 7) visit.

TABLE E2. Distribution of use of controller medications by tertile of tocopherol isoform within children with asthma

Isoform studied in children with asthma and pregnant mothers	Tertile of opposing tocopherol isoform	Without a controller medication (row %)	With a controller medication (row %)
Isoform level in children at the age of 3 y			
α -T	1	33 (57.9)	24 (42.1)
	2	14 (41.2)	20 (58.8)
	3	25 (46.3)	29 (53.7)
γ -T	1	29 (56.7)	22 (43.1)
	2	22 (46.8)	25 (53.2)
	3	21 (44.7)	26 (55.3)
Isoform level in mothers in the second trimester of pregnancy			
α -T	1	32 (51.6)	30 (48.4)
	2	29 (48.3)	31 (51.7)
	3	26 (49.1)	27 (50.9)
γ -T	1	34 (57.6)	25 (42.4)
	2	24 (42.1)	33 (57.9)
	3	29 (49.2)	30 (50.9)

This table covers asthma controller medications in the past year at the mid-childhood visit. Asthma at the mid-childhood visit was limited to subjects who had a physician diagnosis of asthma or wheezing in the past year.

TABLE E3. Comparison of second trimester tocopherol isoform levels (μM) by any prenatal vitamin intake

Second trimester isoform level	No prenatal vitamins (n = 18)	Prenatal vitamins (n = 477)	<i>P</i> value*
Median α -T level (IQR)	63.2 (41.3-100.1)	62.8 (49.4-83.3)	.81
Median γ -T level (IQR)	5.3 (4.5-10.8)	4.3 (3.0-6.4)	.01

**P* value from Wilcoxon rank sum test