Effect of Maternal Smoking on Plasma and Urinary Measures of Vitamin E Isoforms in the First Month after Extreme Preterm Birth

Cosby Stone, Jr, MD, MPH[1], Yunping Qiu, PhD[2], Irwin J. Kurland, MD, PhD[2], James C. Slaughter, DrPH[3], Paul Moore, MD[4], Joan Cook-Mills, PhD[5], Tina Hartert, MD, MPH[1], and Judy L. Aschner, MD[6]

We examined the effect of maternal smoking on plasma and urinary levels of vitamin E isoforms in preterm infants. Maternal smoking during pregnancy decreased infant plasma alpha- and gamma-tocopherol concentrations at 1 week and 4 weeks, with 45% of infants of smokers deficient in alpha-tocopherol at 1 month after birth. *(J Pediatr 2017;■■:■■-■■).*

Vitamin E consists of 8 different antioxidant isoforms with potentially differing contributions to diseases with pathobiology centering on oxidative stress or damage. Two of these isoforms predominate in the human diet and body. The most abundant tissue vitamin E isoform, alpha-tocopherol, has been hypothesized to have a beneficial role in dampening the inflammatory cascade in multiple conditions associated with prematurity, such as bronchopulmonary dysplasia,[1-4] retinopathy of prematurity,[5-9] and intraventricular hemorrhage.[10,11] The next most common isoform, gamma-tocopherol, is not as well studied in neonates. Although known to be a potent antioxidant, gamma-tocopherol appears to be proinflammatory in some disease states, and modifies the protective benefit of alpha-tocopherol.[12-14]

Alpha-CEHC [2,5,7,8-tetramethyl-2-(2'-carboxyethyl)-6-hydroxychroman] and gamma-CEHC [2,7,8-trimethyl-2-(beta-carboxyethyl)-6-hydroxychroman] are the main urinary metabolites of alpha- and gamma-tocopherol, respectively.[15-18] Maternal smoking has been demonstrated to affect the amount of tocopherols present in umbilical cord blood samples, and is postulated to affect the infant's ability to handle oxidative stress.[19] Measurement of these molecules in neonates has previously required a blood draw for plasma or whole blood, which poses technical and medical challenges in small preterm infants. In this study, we examined the effects of maternal smoking on levels of tocopherols and their urinary metabolites in preterm infants in the first postnatal month. We also sought to determine whether urinary and plasma levels of alpha- and gamma-tocopherol and their respective metabolites correlate with one another in the first weeks after birth. This is important for both research and clinical purposes, because it would reduce the need for blood draws to measure these molecules, and facilitate multiple, longitudinal assessments of tocopherol metabolites in research studies.

Alpha-CEHC	2,5,7,8-tetramethyl-2-(2'-carboxyethyl)-6-hydroxychroman
CC	Correlation coefficient
Cr	Creatinine
Gamma-CEHC	2,7,8-trimethyl-2-(beta-carboxyethyl)-6-hydroxychroman
GC	Gas chromatography
MS	Mass spectroscopy
TPN	Total parenteral nutrition

Methods

We studied 41 preterm infants with available blood and urine samples obtained at either 1 week after birth or 1 month after birth to evaluate correlations between plasma alpha- and gamma-tocopherol and their primary urinary metabolites, alpha- and gamma-CEHC. In addition, we analyzed urinary concentrations of F2-isoprostanes, a biomarker of oxidative stress. We had available blood samples at both time points for 30 preterm infants, and urine samples at both time points for 32 infants.

Paired blood and urine samples from the same day were obtained from each infant, although not all infants were able to provide samples at both time points. The first collection occurred at a median of postnatal day 6 and the second at a median of postnatal day 27.

Blood and urine samples from 2 time points were obtained from preterm infants born at <29 weeks' gestational age enrolled in the Improving Prematurity-Related Respiratory Outcomes at Vanderbilt cohort (U01 HL101456), part of the multicenter Prematurity and Respiratory Outcomes Program (PROP; U01 HL101794), a prospective, observational birth cohort of preterm infants born at <29 weeks' gestation. The infants in this study were enrolled at Vanderbilt between September 2011 and December 2013. Specimens for biomarker analysis were collected in the first month after birth with the goal of identifying mechanisms that predict pulmonary outcomes, such as bronchopulmonary dysplasia and respiratory morbidity during the first year after birth. The methods and descriptions of the PROP study have been published

From the [1]Division of Allergy, Pulmonary, and Critical Care Medicine, Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN; [2]Department of Medicine, Stable Isotope and Metabolomics Core Facility, Diabetes Center, Albert Einstein College of Medicine, Bronx, NY; [3]Department of Biostatistics; [4]Division of Allergy, Immunology, and Pulmonary Medicine, Department of Pediatrics, Vanderbilt University School of Medicine, Nashville, TN; [5]Division of Allergy-Immunology, Department of Medicine, Northwestern University School of Medicine, Chicago, IL; and [6]Division of Neonatology, Department of Pediatrics, Albert Einstein College of Medicine and the Children's Hospital at Montefiore, Bronx, NY

Funded by the National Heart, Lung, and Blood Institute (T32 HL87738 to C.S., K24 AI 077930 to T.H., and U01HL101456 to J.A. and P.M.). Support was also provided by the National Center for Advancing Translational Sciences (Clinical and Translational Science Award UL1TR000445). The authors declare no conflicts of interest.

https://doi.org10.1016/j.jpeds.2017.12.062

previously[20-23] and are available at https://clinicaltrials.gov/ct2/show/NCT01460576. Maternal smoking during pregnancy was assessed via standardized questionnaires and chart review, limited to "yes/no" data, without further quantification of the amount of smoking exposure.

Quantification of tocopherol isoforms was performed using gas chromatography–mass spectrometry (GC-MS). The methodology is described in detail in the **Appendix** (available at www.jpeds.com).

Statistical Analyses

Continuous variables were summarized using the median and 25th and 75th percentiles with interquartile range (IQR), and categorical variables were summarized using percentages. The Spearman rank correlation was used to estimate bivariate associations between all pairwise combinations of alpha- and gamma-tocopherol measured in the urine and the plasma. The Pearson test was used to test differences among categorical variables. The Wilcoxon signed-rank test for paired data was used to detect any significant change in tocopherol levels from the first to the second collection time. The Wilcoxon rank-sum test (2 groups) or the Kruskal–Wallis test (more than 2 groups) was used for comparisons across independent groups (eg, smokers vs nonsmokers). Rank methods were used to mitigate the impact of outliers and alleviate the need to consider transformations. Because we report the results of all associations tested, we did not make any formal corrections for multiple comparisons.

Results

The overall maternal and infant characteristics of our cohort with stratification by maternal smoking during pregnancy are reported in **Table I**. There are no significant between-group differences in any of the characteristics studied. We further stratified the same maternal and infant characteristics by maternal race, and likewise noted no significant differences between the groups (**Table II**; available at www.jpeds.com).

Between 1 week and 1 month after birth, the median plasma alpha-tocopherol concentration decreased in our preterm subjects from a median of 22 μmol/L (IQR, 17-28 μmol/L) to 19 μmol/L (IQR, 14-26 μmol/L) (n = 30 paired samples; $P = .02$, Wilcoxon signed-rank rest) and median plasma gamma-tocopherol concentration decreased from 6.4 μmol/L (IQR, 5.3-10.1 μmol/L) to 2.7 μmol/L (IQR, 1.9-5.0 μmol/L) ($P < .001$,

Table I. Clinical and laboratory characteristics of maternal–infant pairs

Characteristic	Overall population (n = 41)	Nonsmoking mother during pregnancy (n = 26)	Smoking mother during pregnancy (n = 15)	*P* value, smoking vs nonsmoking)
Maternal characteristics				
Maternal age, y, median (IQR)	25 (22-29)	25 (22-29)	24 (21-30)	.58
Maternal race/ethnicity, n (%)				
Black	11 (27)	8 (31)	3 (20)	.45*
White	30 (73)	18 (69)	12 (80)	
Hispanic	0 (0)	0 (0)	0 (0)	
Maternal perinatal health factors, n (%)				
Diabetes	2 (5)	0 (0)	2 (13)	.06*
Hypertension	11 (27)	7 (27)	4 (27)	.99*
Asthma	1 (2)	0 (0)	1 (7)	.18*
Smoking during pregnancy	15 (37)	0 (0)	15 (100)	
Infant characteristics				
Infant gestational age, wk, median (IQR)	26 (25-26)	26 (25-26)	26 (25-27)	.58
Infant birth weight, g, median (IQR)	790 (680-930)	786 (698-881)	790 (614-930)	.76
Infant sex female, n (%)	11 (27)	7 (27)	4 (27)	.99*
Infant on TPN at 1 wk of life, n (%)	41 (100)	26 (100)	15 (100)	1
Infant on TPN at 1 mo of life, n (%)	23 (56)	13 (50)	10 (67)	.3
Sample collection, time point 1 (day of life), median (IQR)	6 (5-8)	6 (5-7)	6 (5-8)	.89
Sample collection, time point 2 (day of life), median (IQR)	27 (26-28)	27 (26-28)	26 (26-28)	.53
Plasma tocopherol values, μmol/L, median (IQR)				
Plasma alpha-tocopherol at 1 wk of life	22 (17-28)	27 (21-33)	19 (16-20)	**.007**
Plasma alpha-tocopherol at 1 mo of life	19 (14-26)	22 (16-28)	14 (7-17)	**.002**
• *P* value (comparing 1 wk to 1 mo of life)	**.02**			
Plasma gamma-tocopherol at 1 wk of life	6.4 (5.3-10.1)	8.6 (5.9-10.6)	5.7 (5.3-7.2)	.095
Plasma gamma-tocopherol at 1 mo of life	2.7 (1.9-5.0)	4.1 (2.7-8.1)	2.0 (1.7-2.7)	**.02**
• *P* value (comparing 1 wk to 1 mo of life)	**<.001**			
Urinary CEHC and isoprostane values, median (IQR)				
Urinary alpha-CEHC at 1 wk of life, nmol/L	3.9 (1.9-9.1)	2.6 (2.1-7.2)	3.7 (1.8-19.0)	.70
Urinary alpha-CEHC at 1 mo of life, nmol/L	11.3 (2.8-19.0)	10.4 (2.2-17.3)	6.3 (0.1-13.3)	.64
• *P* value (comparing 1 wk to 1 mo of life)	.26			
Urinary gamma-CEHC at 1 wk of life, nmol/L	5.9 (2.6-13.1)	5.9 (4.0-12.9)	5.7 (2.1-10.8)	.80
Urinary gamma-CEHC at 1 mo of life, nmol/L	8.2 (3.0-20.8)	9.4 (3.5-14.3)	7.5 (0.7-20.5)	.55
• *P* value (comparing 1 wk to 1 mo of life)	.52			
Urinary F2-isoprostanes at 1 wk of life, ng/mg Cr	4.1 (3.1-10.0)	4.2 (2.7-6.9)	4.1 (3.8-16.3)	.16
Urinary F2-isoprostanes at 1 mo of life, ng/mg Cr	5.6 (3.9-11.2)	6.1 (3.7-10.3)	5.6 (4.4-11.5)	.79

Significant *P* values are in bold type.
CR, creatinine.
*Ais conducted with Pearson test. Comparisons of 1 week to 1 month used the paired Wilcoxon rank-sum test; all others used the Wilcoxon signed-rank test.

CRP 5.5.0 DTD ■ YMPD9695_proof ■ February 2, 2018