



Letter

Association of exhaled nitric oxide with ethnicity and sex in rural Georgia youth

Nitric oxide is produced by the human bronchial epithelium¹ and is associated with airway eosinophilic inflammation seen in patients with asthma and other airway diseases.² Airway inflammation is a characteristic of asthma, along with airway hyperresponsiveness and airway obstruction. Inhaled corticosteroids to treat the inflammation are a mainstay of asthma treatment. Measurement of exhaled nitric oxide (eNO) as a biomarker of eosinophilic inflammation is noninvasive, safe, reproducible, and easy to perform; however, the clinical value of measurements has been limited by imprecise reference ranges. Established reference ranges must account for many variables known to affect eNO values. Some have suggested that a threshold value may be more useful than reference ranges.³

Studies have evaluated the association of eNO and ethnicity and/or race but this information is limited. A 2017 study investigated factors related to eNO in 716 children between the ages of 6 and 19 years from the 2007–2012 National Health and Nutrition Examination Survey (NHANES).⁴ High levels of eNO (defined by American Thoracic Society guidelines as >35 ppb for the ages of 6–11 years and >50 ppb for the ages of 12–19 years) were more frequently found in non-Hispanic black and Hispanic than non-Hispanic white individuals.⁴ Recently, a systematic review⁵ comprising 12 studies of ethnicity and eNO was published. Ten of these studies presented ethnicity as a significant factor in eNO levels. A different study, including 4718 patients from 2007–2010 NHANES, found that eNO was significantly lower in non-Hispanic whites compared with non-Hispanic blacks and Hispanics between the ages of 6 and 19 years. In individuals older than 19 years, there was no difference in eNO according to ethnicity.⁶

Some studies have evaluated sex as well; however, few investigated both ethnicity and sex groups simultaneously in association with eNO levels. In addition, one plausible variable that may affect the association of eNO with ethnicity and sex is exposure to air pollutants, which are typically greater in urban areas. Previous studies have almost exclusively examined urban populations, making our rural study population unique. Further clarification of fixed influential variables could possibly improve the clinical utility of eNO measurements. The purpose of this study was to examine the associations of race and sex with eNO in a representative sample of rural teenage youth with asthma symptoms.

The initial data were collected as part of the Puff City Georgia randomized controlled trial ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study?term=NCT01170676) Identifier: NCT01170676), which tested the effectiveness of a computer-based intervention to improve asthma self-management.⁷ The study surveyed high school students in the single public high school in each of 4 rural counties of Southeastern Georgia. The students reported asthma and asthma symptoms using the Lung Health Survey, a questionnaire composed of questions used in the International Study of Asthma and Allergies in Childhood questionnaire. Collected information includes physician diagnosis of asthma, respiratory symptoms, sex, and race. Exhaled NO was measured per manufacturer's protocol using the NIOX MINO eNO instrument by trained research study staff. The study was approved by the Human Assurance Committee of Augusta University, and permission was provided by the respective school superintendent and principal in each county in Georgia.⁷

Statistical analysis was performed using SAS statistical software, version 9.3 (SAS Institute Inc, Cary, North Carolina), with statistical significance using an α level of .05. Two-factor analysis of variance (ANOVA) was used to examine differences between race and sex groups of the geometric mean eNO. Adjusted least squares geometric means for eNO were calculated within each effect in the 2-factor ANOVA model, including race, sex, and race and sex groups. A Bonferroni adjustment to the overall α level (adjusted $\alpha = 0.05/4 = .0125$) was used to examine post hoc pairwise differences between ethnicity and sex groups.

A total of 353 students participated in this study. Of these, 238 (68.0%) were black, 88 (25.1%) white, and 24 (6.9%) were categorized as other. Three individuals had missing racial information. There were 132 males (37.4%) and 221 females (62.6%). Full study participant characteristics are presented in [eTable 1](#).

The geometric mean eNO for race and sex groups differed significantly ($P < .001$). Black males had significantly higher geometric mean eNO levels than black females ($P < .001$). White or other females were found to have significantly lower geometric mean eNO levels than black males ($P < .001$), black females ($P < .001$), and white or other males ($P < .001$). These data are presented in [Table 1](#).

Our data and prior research help illustrate potential confounders in eNO, which affects its clinical use. When eNO is used, consideration of race and/or ethnicity and sex in conjunction with other confounders may be required. A recent review by Petsky et al⁸ evaluated multiple studies that investigated the benefit of eosinophilic markers in asthma. They found that both adults and pediatric patients experienced significantly fewer exacerbations compared with the control group when treatment was adjusted using eNO; however, use of eNO did not significantly change inhaled corticosteroid dose.⁸ More research on eNO is needed to determine how to

Disclosures: Authors have nothing to disclose.

Funding Sources: Research reported in this publication was supported by the National Heart, Lung, and Blood Institute of the National Institutes of Health under award R01HL092412.

Disclaimer: The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

<https://doi.org/10.1016/j.anai.2018.12.011>

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Table 1
Analysis of Variance Results for Differences in Geometric Mean eNO Levels

Variable	Geometric mean (95% CI)	F	P value
Race			
Black	25.31 (22.71–28.21)	14.61	<.001
White or other	17.45 (14.90–20.43)		
Sex			
Male	30.52 (26.44–35.24)	27.12	<.001
Female	18.89 (16.92–21.09)		
Race and sex			
Black male	33.65 (28.25–40.10)	15.68	<.001
Black female	21.67 (19.04–24.66)		
White or other male	25.06 (19.78–31.76)		
White or other female	13.55 (11.12–16.51)		

Abbreviation: eNO, exhaled nitric oxide.

use it more effectively in the clinical arena for the treatment of asthma and other disease states.

Strengths of this study include (1) a large sample that represents high school students with asthma symptoms in rural settings, (2) the predominately black sample, and (3) inclusion of a biological measure of eosinophilic inflammation (eNO) in addition to the survey measures. Limitations of this study are the multiple self-reported individual characteristics and the inability to assess atopy by skin prick testing or measurement of allergen specific IgE. In addition, these results may only be generalizable to a similar rural and largely black teenage populations. We do not have additional data allowing us to suggest a reason for the race $\times$ sex effect on eNO. Use of eNO to evaluate the effectiveness of asthma treatment may be beneficial, and this study provides additional information on potential confounders in its use.

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eTable 1

Descriptive Statistics for Demographic Characteristics at Baseline Evaluation

Variable	Finding ^a
Race	
White	88 (25.1)
Black	238 (68.0)
Other	24 (6.9)
Black race	
Black	238 (68.0)
White or other	112 (32.0)
Sex	
Male	132 (37.4)
Female	221 (62.6)
Race and sex	
Black male	85 (24.2)
Black female	155 (44.0)
White or other male	46 (13.1)
White or other female	66 (18.7)
Age, mean (SD), y	15.5 (1.0)
eNO, mean (SD), ppb	33.5 (33.5)
eNO, geometric mean (SD), ppb	22.6 (2.4)
eNO category	
Normal (≤ 25 ppb)	207 (59.0)
Moderate (25–50 ppb)	71 (20.2)
High (> 50 ppb)	73 (20.8)

Abbreviation: eNO, exhaled nitric oxide.

^aData are presented as number (percentage) of patients unless otherwise indicated.