Exhaled NO reference limits in a large population-based sample using the Lambda-Mu-Sigma method

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ABSTRACT

Absolute values are used in the interpre-tation of the fraction of exhaled nitric oxide (FeNO), but it has been suggested that equations to calculate reference values may be a practical and clinically useful approach. We hypothesize that the application of the Lambda-Mu-Sigma (LMS) method may improve FeNO reference equations and their interpretation. Our aims were to develop FeNO reference equations with the LMS method and to describe the difference between this method and the absolute fixed cut-offs of the current recommendations. We utilized the United States National Health and Nutrition Examination Surveys 2007–2012 and included healthy individuals with no respiratory diseases and blood eosinophils $<300/mm^3$ ($n = 8,340$). Natural log-transformed FeNO was modeled using the LMS method, imbedded in the gener-alized additive models for location, scale, and shape models. A set of FeNO reference equations was developed. The explanatory variables were sex, age, height, smoking habits, and race/ethnicity. A significant proportion of individuals with normal FeNO given by the equations were classified as having intermediate levels by the current recom-mendations. Further lower predicted FeNO compared with previous linear models was seen. In conclusion, we suggest a novel model for the prediction of reference FeNO values that can contribute to the interpretation of FeNO in clinical practice. This approach should be further validated in large samples with an objective measurement of atopy and a medical diagnosis of asthma and rhinitis.

NEW & NOTEWORTHY Novel reference equations and fraction of exhaled nitric oxide (FeNO)-predicted values to improve interpretation of FeNO in clinical practice are presented. These may increase the accuracy of ruling out airway inflammation in patients with asthma or suspected asthma.

asthma; exhaled nitric oxide; healthy; reference values

INTRODUCTION

Measurement of the fraction of exhaled nitric oxide (FeNO) is now recognized as an accurate, reproducible, and noninvasive diagnostic test for airway disease and is increasingly used in clinical practice (6). FeNO primarily signals airway inflammation that is triggered by IL-4 and IL-13 (3). However, FeNO is influenced by several individual factors, including age, height, sex, atopy, and smoking habits (15). This is an issue that makes the adequate interpretation of this diagnostic test more difficult (39).

A diagnostic test result can be presented in different ways, for example, in absolute values. However, absolute values are not commonly used in lung function tests, but in the case of FeNO, the most recent American Thoracic Society (ATS) guidelines have suggested their use for interpretation (12). Specifically, the guidelines state that, in steroid-naïve subjects, a FeNO of 25 parts per billion (ppb) (20 ppb in children <12 yr old) indicates a low likelihood of eosinophilic inflammation and corticosteroid responsiveness, whereas a FeNO of >50 ppb (>35 ppb in children) indicates a high probability of eosinophilic airway inflammation. The intermediate FeNO range of 25–50 ppb (20–35 ppb in children) should be interpreted with consideration of the clinical context. However, the guidelines recognize that these fixed cutoffs are weak recommendations with low quality of evidence (12). One of the solutions proposed to consider the limitations of absolute values has been a "personal best" value for FeNO (37). Although this is a strong approach if the objective is to monitor FeNO, this method cannot be used for the initial assessment of FeNO in a patient. Furthermore, the personal best values were shown to be close to, at the time, published reference values (27). Thus, the use of equations to calculate reference values may be a more practical and clinically useful approach (24), as recently shown using multiple linear regression models (41). However, FeNO does not follow a normal distribution in the healthy population (15), and the lifetime evolution of FeNO (17), and the large variation of FeNO in the general population, contributes to the fact that such models have poor predictive capability (36). Similar issues were identified in the interpretation of lung function with spirometry (40), which has led to the development of a new approach by the Global Lung

Initiative, a European Respiratory Society (ERS) task force. The Global Lung Initiative used very robust statistical regression methods (38), the lambda-mu-sigma (LMS) method (9), to model lung function adjusted for sex, height, and ethnicity. The resulting powerful reference equations have supported the use of z-scores and percentiles for the classification of spirometric parameters (31).

We hypothesize that the application of the LMS method may improve the quality of reference equations for FeNO, and the subsequent interpretation of this marker, in a healthy population. Our aims were *1*) to develop reference equations for FeNO with the LMS method by using data from the National Health and Nutrition Examination Survey (NHANES) (2007–12), *2*) to describe the difference between the LMS model and the fixed cutoffs of the 2011 ATS recommendations, and *3*) to compare with previously published multiple linear regression models.

METHODS

Study population. The study population was composed of individuals who participated in the NHANES. The NHANES is a nationally representative survey of the noninstitutionalized United States civilian population conducted to evaluate health and nutritional status. In each survey, participants are selected using a complex stratified multistage, probability-based sampling design, and information is collected by standardized household interviews, physical examinations, and testing of biological samples (10, 13, 19). More details are available at: https://www.cdc.gov/nchs/nhanes.htm. For this analysis, data from the NHANES 2007 to 2012 were used. From 30,442 participants aged 6 to 80 yr, we have excluded subjects with asthma, emphysema, or chronic bronchitis (n = 4,938), without valid or reproducible FeNO measurements (n = 9,795), and with a blood eosinophil count >300/mm^3 (n = 7,011). Thus, we included respiratory healthy individuals with a normal blood eosinophil count (n = 8,340).

Variables. FeNO measurements were performed with NIOX MINO (Aerocrine, Solna, Sweden), following ATS/ERS recommendations (4). In brief, participants were asked to empty their lungs, to place their mouth on the analyzer's mouthpiece, and to fill their lungs with NO-free air. Then, they were asked to blow out all of their air at a constant flow of 50 ml/s, for 10 s if height was at or above 130 cm and 6 s for those below 130 cm. At least two reproducible measurements were obtained, defined by if either or both of the first two valid FENO measurements were below 30 ppb and the measurements were within 2 ppb of each other or if both measurements were over 30 ppb and within 10% of each other. The following aspects were considered before FeNO measurements: breathing problem requiring oxygen, problem taking deep breaths, smoked the last hour before the measurement, strenuous exercise in the last hour, eating and drinking in the last hour, eating nitrate-rich vegetables and nitrite-rich meats in the last three hours, and cough, cold, or respiratory illness in the past seven days. More details can be found online (https://wwwn.cdc.gov/Nchs/Nhanes/2011-2012/ENX_G.htm).

The mean of two reproducible FeNO measurements was considered; a value of 3.5 ppb was used if the measurements were below the detection limit of the device (5 ppb, n = 1,690). Age was calculated with one decimal point as the difference between date of birth and date of examination in both data sets. The categories in the race/ethnicity groups were based on the NHANES classification (8). Four groups were created: non-Hispanic white; non-Hispanic black; Mexican-American (including other Hispanics), and other (multi-racial). An active smoker was defined as a positive answer to both of the following questions: "Have you smoked ≥100 cigarettes during your lifetime?" and "Do you now smoke cigarettes?", and a never smoker was defined as a negative answer to the same questions. Since these questions were only asked in subjects aged ≥16 yr old, we considered subjects with an age <16 yr never smokers as well. We have previously shown that former smokers (positive answer to the first question and negative to the second) have a FeNO similar to never smokers, if not presently exposed (18). Thus, only current smoking was used as explanatory variable in the full data set. However, in the alternative data set, only never smokers were included since former smokers seem to be more passively exposed to cigarette smoke (18).

Statistical analysis. Categorical variables are presented as absolute frequencies and proportions. Mean and standard deviation are presented for continuous variables. FeNO is presented using geometric means, given its right-skewed distribution, and was transformed using the natural logarithm (ln), with 95% confidence interval (95% CI). To allow the modeling of lnFeNO values and more than one explanatory variable, we applied the LMS method (9), imbedded in the generalized additive models for location, scale, and shape (GAMLSS) models. GAMLSS is a semiparametric regression-type of statistical model, which is highly flexible as it relaxes the traditional distributional assumptions about normality to include even highly skewed and kurtotic distributions (33). The parameters L, M, and S of the distribution are interpreted as a participant's expected mean (M) and additionally for a participant's expected coefficient of variation (S) and skewness (L) (31). A more detailed description of the statistical methodology is described elsewhere (31, 40). FeNO prediction models were derived for men and women, respectively, with a fitting spline curve from the NHANES, with the explanatory variables: height, age, smoking habits (smoker vs nonsmoker), and ethnicity. After using the models to predict the FeNO value in a given individual, it is possible to calculate the upper limit of normal at the 95th percentile (ULN 95th) and the corresponding z-score, as follows: ULN 95th = $\exp(\ln(M) + \ln(1 + 1.645.L.S)/L)$ and z-score = $[(\text{measured}/M)^L - 1]/(L.S)$ (29). Essentially, the general form is a linear regression equation with an age-specific correction in the form of the age-spline. Model selection was based on generalized Akaike information criterion (GAIC), an index designed to be an unbiased estimator of the information regarding the model fitting. The model with the lowest value of GAIC is considered as having the best fit (1) and was selected as the final model. Worm plots and normal Q-Q visual inspection were additionally used to assess distribution and density plots of residuals as a function of age. The GAMLSS package implemented in R statistical software (version 3.1.1) was used for the analysis. All other statistical analyses were performed using IBM SPSS Statistics, version 23.0. A P value of <0.05 was considered as statistically significant. The proportion of observed FeNO greater than the predicted ULN 95th was calculated.

We have compared the LMS model with two previously published models (36, 41) by generating the predicted FeNO and the ULN 95th for 4 example subjects, 2 men and 2 women, using the age breakpoints previously described (17) and with the mean height in each age strata.

Ethics. The NHANES studies were approved by the ethics committee of The National Center for Health Statistics Ethics Review Board. All participants or parents/legal representatives signed the consent form.

RESULTS

Subject characteristics. We have included 8,340 subjects from the NHANES 2007–2012, from which 1,802 (22%) were subjects aged <16 yr. Two sets of models for FeNO were derived, one with the whole sample and one with a subgroup of never smokers (n = 6,101). The characteristics of the included participants are shown in Table 1.

The final model, based on the whole sample and including all explanatory variables, showed a better fit ($GAIC_{males}$ = 6,874 and $GAIC_{females}$ = 7,135, the lower the better) than models that included only age and height ($GAIC_{males}$ = 7,177 and $GAIC_{females}$ = 7,400), and a similar fit compared with a model including age, height, and smoking status ($GAIC_{males}$ = 6,915

Table 1. *Description of participants included in the derivation of reference equations for FeNO*

	Men			Women		
Whole sample	Total (n = 4,001)	<16 yr (n = 851)	≥16 yr (n = 3,150)	Total (n = 4,339)	<16 yr (n = 949)	≥16 yr (n = 3,388)
Age range, mean (min–max)	36.5 (6–79)	10.5 (6–15)	43.4 (16–79)	36.2 (6–79)	10.5 (6–15)	43.6 (16–79)
Height, cm	168.8 (14.6)	147.0 (18.0)	174.7 (7.8)	157.7 (11.6)	145.0 (15.0)	161.2 (7.1)
BMI, kg/cm^2	26.2 (6.5)	19.6 (4.4)	28.0 (5.7)	26.7 (7.5)	20.3 (5.2)	28.4 (7.1)
Ethnicity, n (%)						
Non-Hispanic white	1,587 (40)	300 (35)	1,287 (41)	1,656 (38)	293 (31)	1,363 (40)
Non-Hispanic black	821 (20)	160 (19)	661 (21)	970 (22)	233 (24)	737 (22)
Hispanic	1,242 (31)	317 (37)	925 (29)	1324 (31)	340 (36)	984 (29)
Other/multiracial	351 (9)	74 (9)	277 (9)	389 (9)	85 (9)	304 (9)
Smoker, n (%)	495 (12)	NA	495 (16)	373 (9)	NA	373 (11)
Hay fever, n (%)	396 (10)	73 (9)	323 (10)	492 (11)	79 (8)	413 (12)
FeNO (ppb), geo. mean (Q1–Q3)	12.9 (8.5–19.5)	9.1 (6.5–13.0)	14.1 (10.0–21.0)	11.1 (7.5–16.0)	9.0 (6.5–12.5)	11.7 (8.0–17)
Never smokers	Total (n = 2,676)	<16 yr (n = 851)	≥16 yr (n = 1,825)	Total n = 3,389	<16 yr n = 949	≥16 yr n = 2,440
Age range, mean (min–max)	29.8 (6–79)	10.5 (6–15)	38.8 (16–79)	32.8 (6–79)	10.4 (6–15)	41.5 (16–79)
Height, cm	165.9 (17.6)	147.0 (18.0)	174.7 (7.8)	156.4 (12.3)	145.0 (15.0)	160.8 (7.2)
BMI, kg/cm^2	25.2 (6.6)	19.6 (4.4)	27.8 (5.8)	26.1 (7.6)	20.3 (5.2)	28.3 (7.2)
Ethnicity, n (%)						
Non-Hispanic white	1,006 (38)	300 (35)	706 (39)	1,146 (34)	293 (31)	853 (35)
Non-Hispanic black	555 (21)	160 (19)	395 (22)	769 (23)	232 (24)	537 (22)
Hispanic	874 (33)	317 (37)	557 (30)	1,126 (33)	339 (36)	787 (32)
Other/multiracial	241 (9)	74 (9)	167 (9)	348 (10)	85 (9)	263 (11)
Hay fever, n (%)	258 (10)	73 (9)	185 (10)	370 (11)	79 (8)	291 (12)
FeNO (ppb), geo. mean (Q1–Q3)	12.9 (9.5–19.5)	9.1 (6.5–13.0)	15.1 (10.5–21.5)	11.3 (7.5–16.0)	9.0 (6.5–12.5)	12.5 (8.5–17.5)

Values are means (SD). BMI, body mass index; FeNO, fraction of exhaled nitric oxide; NA, not available; ppb, parts per billion; Q1/3, Quartile 1/3.

and $GAIC_{females}$ = 7,125). The model derived from the never-smokers sample, that included the variables height, age, and race/ethnicity, showed the best fit of all tested models ($GAIC_{males}$ = 4,442 and $GAIC_{females}$ = 5,444).

The models and their variables are shown in Table 2.

As an example, for a 19-yr-old woman, 165 cm tall, non-smoker, Caucasian with no hay fever in the past 12 mo, with an observed FeNO value of 35 ppb, one would first retrieve the Mspline and Sspline from the lookup tables (available online), in this case, −0.00209 and 0.025124, respectively. Next, the predicted FeNO is calculated as follows: ln(FeNO) predicted = M = exp(−1.60112 + 0.41884 × ln(165) + 0.10275 × ln(19) + 0. 00209) = exp (2.37) = 10.7 ppb, the ULN 95th = exp(ln(1 + 1.645 × 0.2318 × 0.235)/0.2318 + ln(10.8)) = 22.2 ppb and the z-score = ((ln(35)/ln(10.14))$^{0.2318}$ − 1)/(0.2318 × 0.235) = 2.08. Thus, the observed FeNO is to be considered elevated.

Effects of explanatory variables. When compared with healthy, nonsmoking, nonatopic, non-Hispanic whites, smoking reduces FeNO values by 32.6% (95% CI 30.5%–34.9%) in men and by 37.5% (95% CI 35.2%–39.7%) in women. In addition, the included races/ethnicities have proportionally higher FeNO compared with non-Hispanic whites; male non-Hispanic blacks have FeNO increased by 3.6% (95% CI 1.3%–5.8%), male Hispanics by 3.3% (95% CI 1.3%–5.3%), and male other/multi-racial by 18.4% (95% CI 15.3%–21.5%).

Table 2. *Regression coefficients for ln(FeNO) for calculating M, S, and L for men and women, respectively*

	Coefficients								
	M			S			L		
		Men	Women		Men	Women		Men	Women
Never smokers (n = 6,065)									
Intercept	a0	−2.3332	−1.6649	p0	−0.9590	−1.0151	q0	−0.4836	−0.5863
Height	a1	0.5694	0.4333						
Age	a2	0.1041	0.1013	p1	−0.1774	−0.1645	q1	0.4517	0.4129
Non-hispanic black	a3	0.0225	0.0339	p2	0.1108	0.1306			
Hispanic	a4	0.0426	0.0227	p3	−0.0678	0.0797			
Other/multiracial	a5	0.0767	0.0489	p4	−0.0535	0.0375			
	+	Mspline	Mspline	+	Sspline	Sspline	+	Lspline	Lspline
Whole sample (n = 8,340)									
Intercept	a0	−2.1279	−1.60112	p0	−1.00682	−1.0175	q0	−1.00682	−1.01752
Height	a1	0.5278	0.41884						
Age	a2	0.1021	0.10275	p1	−0.15394	−0.1544	q1	0.42066	0.42892
Smoker	a3	−0.1955	−0.23005	p2	0.27337	0.2778			
Non-Hispanic black	a4	0.0271	0.02752	p3	0.08516	0.0972			
Hispanic	a5	0.0555	0.03019	p4	−0.06813	0.0612			
Other/multiracial	a6	0.0913	0.04835	p5	−0.09938	0.0228			
	+	Mspline	Mspline	+	Sspline	Sspline	+	Lspline	Lspline

Contributions of splines must be added to the calculated values; they are available in look-up tables. FeNO, fraction of exhaled nitric oxide; L, skewness; ln, natural logarithm; M, expected mean; S, expected coefficient of variation.

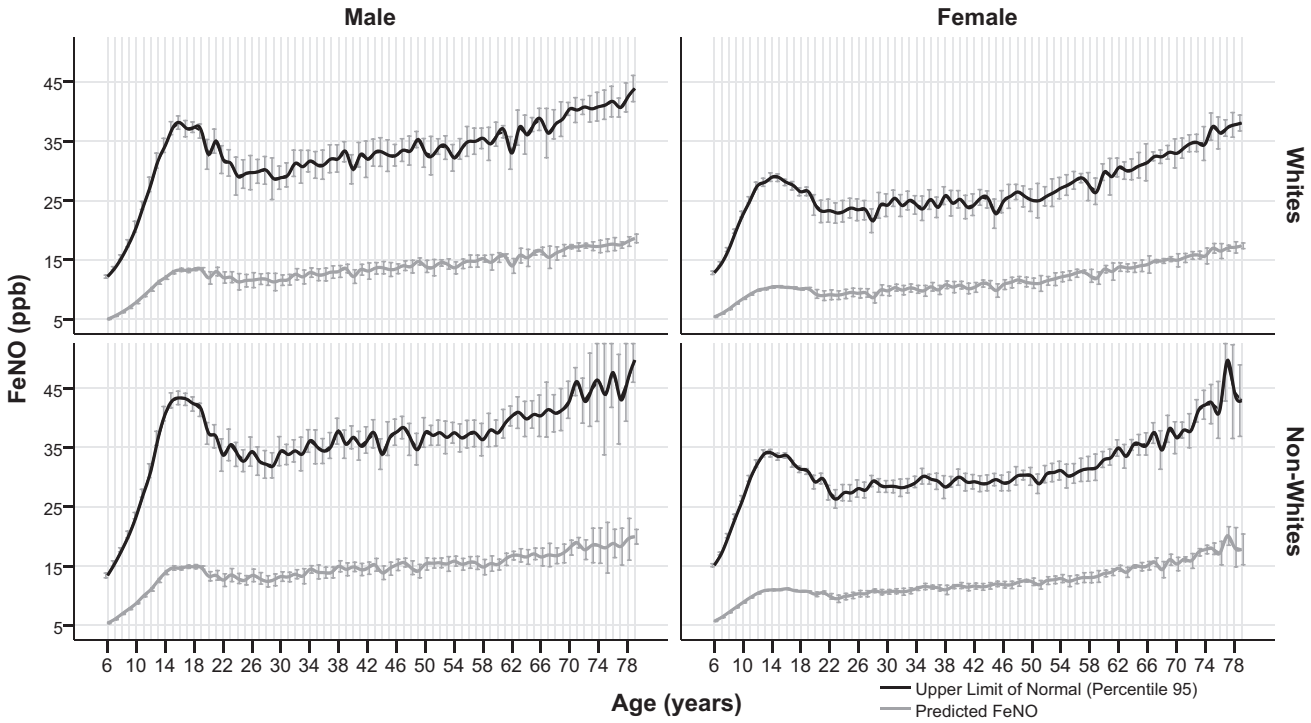

Fig. 1. Predicted values and upper limit of normal at the 95th percentile for fraction of exhaled nitric oxide (FeNO) obtained with the model created using the lambda-mu-sigma method and stratified by sex (male vs. female) and race (non-Hispanic whites vs. others). ppb, parts per billion.

Female non-Hispanic blacks have FeNO increased by 3.6% (95% CI 1.4%–5.7%), and female other/multi-racial by 4.3% (95% CI 1.4%–7.2%). The overall model for predicted FeNO and ULN 95th in the entire age range, stratified by gender, is shown in Fig. 1.

Furthermore, men have slightly higher FeNO values than women, 15.7 ppb (95% CI 8.5–19.5) versus 13.3 ppb (95% CI 7.5–16.0), independent of age and race/ethnicity (Table 3). This difference was consistent in adults, whereas there was no significant difference in FeNO between boys and girls among children less than 12 yr old, 9.3 ppb (95% CI 5.5–11.0) versus 9.8 ppb (95% CI 6.0–12.0). The ULN 95th for FeNO is also significantly lower in boys aged <12 yr old compared with adult men, 19.5 ppb (95% CI 15.3–23.2) versus 36.3 ppb (95% CI 32.7–40.8), whereas this was not seen in women 22.1 ppb (95% CI 17.5–26.1) versus 29.7 ppb (95% CI 27.0–32.9).

Comparison with recommended absolute cutoffs. Figure 2 shows the proportion of individuals with FeNO <ULN 95th or ULN 90th and >ULN 95th or ULN 90th in the groups with low FeNO (<25 ppb, <20 ppb in children), intermediate FeNO (25–50 ppb, 20–35 ppb in children), and high FeNO (>50 ppb, >35 ppb in children), as defined by the ATS guidelines (12). There were 743 subjects classified in the intermediate FeNO category (25–50 ppb in adults and 20–35 ppb in children), where the highest proportion of disagreement between the classification of the 2011 recommendations occurs with 34% (<ULN 90th) or 63% (<ULN95th) being classified as having normal FeNO. The overall Cohen's unweighted kappa (95% CI) is 0.67 (0.65–0.69).

Comparison with multiple linear regression models. The comparison of the LMS model with the linear models published by See et al. (36) and Torén et al. (41) is shown in Table 4. The model from Torén et al. was derived from a sample of individuals with an age range of 25–75 yr, and thus no values for adolescents are presented. The model from See et al. does not allow for the calculation of an individual ULN.

Table 3. *Predicted FeNO and ULN by using the LMS method applied on NHANES subjects, stratified by age and sex*

	Men			Women		
	<12 yr	≥12 yr	All ages	<12 yr	≥12 yr	All ages
Never-smokers sample	(*n* = 547)	(*n* = 2,140)	(*n* = 2,687)	(*n* = 629)	(*n* = 2,785)	(*n* = 3,414)
Observed values, ppb‡	9.3 (5.5–11.0)	14.7 (10.5–21.0)	12.9 (8.5–19.5)	9.8 (6.0–12.0)	12.1 (8.5–17.0)	11.3 (7.5–16.0)
Predicted values, ppb‡	7.4 (6.2–8.7)	14.8 (13.5–16.3)	12.8 (11.7–15.9)	7.3 (6.7–8.8)	12.0 (10.7–13.4)	11.1 (9.99–12.8)
Predicted ULN 95th, ppb‡	19.1 (15.8–23.2)	37.2 (34.0–40.7)	32.5 (30.9–39.6)	21.5 (17.8–25.5)	30.2 (27.4–33.0)	28.4 (26.1–32.2)
Whole sample	(*n* = 547)	(*n* = 3,439)	(*n* = 3,986)	(*n* = 629)	(*n* = 3,679)	(*n* = 4,308)
Observed values, ppb‡	9.3 (5.5–11.0)	16.7 (9.5–20.5)	15.7 (8.5–19.5)	9.8 (6.0–12.0)	13.9 (8.0–17.0)	13.3 (7.5–16.0)
Predicted values, ppb‡	7.4 (6.1–8.7)	14.3 (12.9–16.2)	13.4 (10.5–15.9)	7.8 (6.6–9.0)	11.8 (10.5–13.2)	11.2 (9.9–12.8)
Predicted ULN 95th, ppb‡	19.5 (15.3–23.2)	36.3 (32.7–40.8)	34.0 (28.3–40.0)	22.1 (17.5–26.1)	29.7 (27.0–32.9)	28.6 (25.8–32.4)

FeNO, fraction of exhaled nitric oxide; LMS, lambda-mu-sigma; ppb, parts per billion; Q1/3, quartile 1/3; ULN 95th, Upper limit of normal (95th percentile).
‡Geometric mean (Q1–Q3).

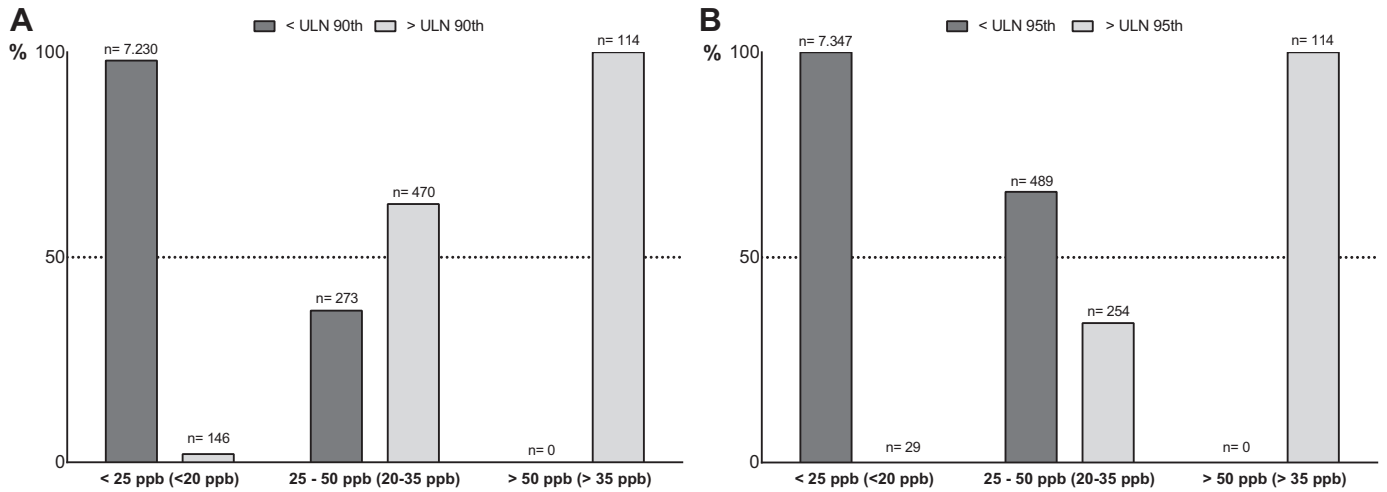

Fig. 2. Proportion of individuals with predicted fraction of exhaled nitric oxide (FeNO) <upper limit of normal at the 90th percentile (ULN 90th) and >ULN 90th (*A*) and <ULN 95th and >ULN 95th (*B*) classified as having low FeNO, intermediate FeNO, and high FeNO, according to the 2011 American Thoracic Society recommendations (12). The absolute number of subjects in each category is presented above each bar. There were 89% of subjects in the low FeNO group, 10% in the intermediate group, and 1% in the high group. ppb, parts per billion.

DISCUSSION

In this study, we present reference equations for FeNO, created with the LMS method, a robust methodological and statistical approach. The explanatory variables of the equations, separate for men and women, were age, height, smoking habits, and race/ethnicity, which are the most commonly reported individual factors influencing FeNO (15). A significant proportion of individuals with FeNO below the ULN given by the LMS models are classified as having intermediate levels by the 2011 ATS recommendations (12).

The derived equations were created using the LMS method, which has previously been used in the construction of growth charts in children (9). More recently, it was used in new reference equations for lung function, which provided predicted values and age-corrected lower limits of normal for spirometry in a wide range of ages (31). Furthermore, this method allows for the modeling of the expected mean, accounting for complex effects of explanatory variables on the dependent variable with splines, which, in turn, allows for the dependent variable to vary smoothly (i.e., nonlinearly) as a function of an explanatory variable. Thus, the main advantage of this method was the development of a continuous, smooth fit over a wide age range (31, 32). Given the nonlinear effect of age on FeNO, as previously described (17), and the previous use of statistical models that does not account for the nonlinearity of the dependent variable (15, 36), it seemed reasonable to apply the LMS method on FeNO.

The formation of NO is a complex and energy-consuming biological process (3) that is seen already at birth (25), suggesting that airway NO formation is important in humans and, consequently, should normally be under strict biological control. The origin of NO in the airway epithelium, produced by inducible NO synthase, indicates that the total surface area of the airway mucosa will be an important determinant of FeNO. Indeed, the airway diffusing capacity for NO, which theoretically should be dependent on the airway mucosal surface area, has been shown to correlate with the anatomic dead space volume in healthy children (28). Thus, it is logical to assume that age, height, and gender are important factors when evaluating FeNO values, as seen for lung function parameters (17). This has also been demonstrated previously with linear regression models for FeNO (11, 22, 27, 41).

However, the effect of race on FeNO is less established (34); current data are inadequate to allow conclusions concerning people of different genetic backgrounds. The practice of "race correction" or "ethnic adjustment" of predicted lung-function values derived from reference equations is still recommended and may be the best approximation for current estimation of a "normal" lung function in a well-defined population (35). Regarding FeNO, despite some evidence on the influence of race/ethnicity in specific populations (16, 20), it is still difficult to establish a common ground of understanding, or at least one that can be easily validated when comparing populations (15).

Table 4. *Comparison of predicted FeNO and ULN 95th of the LMS model with previously published models*

	See et al. 2011 (36)		Toren et al. 2017 (41)		LMS Model	
	Predicted	ULN 95th	Predicted	ULN 95th	Predicted	ULN 95th
Male, 15.5 y, 172 cm	13.9	NA	NA	NA	11.4	24.1
Male, 61.3 y, 173 cm	16.6	NA	18.9	42.8	14.3	30.3
Female, 13.9 y, 160 cm	11.0	NA	NA	NA	10.0	20.7
Female, 44.5 y, 162 cm	12.7	NA	13.7	29.0	12.0	25.0

All subjects were considered nonsmokers and nonatopics. In Toren et al., atopy was objectively confirmed with Phadiatop. Values in parts per billion. FeNO, fraction of exhaled nitric oxide; LMS, lambda-mu-sigma; NA, not available; ULN 95th, upper limit of normality (95th percentile).

Cigarette smoking has long been known to reduce FeNO in healthy subjects (30), and there are multiple mechanisms proposed to explain this effect on FeNO. For example, lower levels of IFN-γ and IFN-γ-expressing cells in the airways of smokers than in nonsmokers (21, 26, 42), and the reduction of L-arginine bioavailability in the mucosa through the upregulation of epithelial arginase-1 (5), may explain reduced FeNO in present smokers (18).

The LMS models' predicted values were slightly lower than the observed values in both men and women, which may be due to the skewed distribution of FeNO. However, these differences were not statistically significant. The ULN was higher in men than women, as expected (17). It is of note that the ULN of FeNO in children below the age of 12 yr was similar to the proposed fixed cutoffs of 20 ppb for the likely absence of eosinophilic airway inflammation and benefit of inhaled corticosteroids (12), 19.5 ppb in boys and 22.1 ppb in girls, although the predicted FeNO is dependent on age (or height) also in this age group (14). However, this was not the case in adults or when considering the overall population, with limits above the suggested 25 ppb: 36.3 ppb in men and 29.7 ppb in women. This difference was statistically significant and is probably due to the known changes in FeNO throughout the human age range. We have previously shown that there are two significant points of change in the FeNO evolution throughout the human age range, at the ages of ~16 and 61 yr in men and 14 and 45 yr in women, thus, both occurring after the age of 12 yr (17). The fixed cutoffs (12) do not fully account for these changes, whereas the proposed models do. This fact is shown by the poor agreement between the ULN and the recommended categories of low FeNO (<25 ppb, <20 ppb in children), intermediate FeNO (25–50 ppb, 20–35 ppb in children), and high FeNO (>50 ppb, >35 ppb in children), regardless of using the 90th or 95th percentile. The high cutoffs of 50 ppb or 35 ppb (children) in the ATS guidelines (12) are recommended to be used for ruling in eosinophilic airway inflammation and a high likelihood of a clinical response to inhaled corticosteroids. However, it may be more important to be able safely to rule out airway inflammation in patients with asthma or suspected asthma, and we found that 63% of individuals below ULN 95th were classified as having an intermediate FeNO, which may impair the initial assessment of patients with respiratory symptoms.

The LMS models presented in this study show lower predicted values and ULN when compared with the models by See et al. and Torén et al. This may be expected, given the fact that linear models may not be the best approach to accommodate the nonlinear evolution of FeNO with age, and the chosen age points for the example subjects were at the ages where the largest deviation between the models is expected. In addition, the LMS model should theoretically be better at dealing with the skewed distribution of FeNO in each age stratum. Furthermore, the advantage of the LMS model is that predicted values and ULNs can be calculated from a single model, whereas See et al. (36) had two different models for children aged 6–11 yr and adults 12–80 yr, respectively. As far as we are aware, this is the first description of predicted FeNO and ULN in the whole age range of 6–80 yr.

One limitation of the present study is the lack of reliable information on atopy. Future development of FeNO reference equations should include an objective marker of IgE sensitization. However, it may be difficult to capture the effect of atopy in a dichotomous fashion since atopy may result in an increase in FeNO of anywhere between 0 and more than 100 ppb, depending on the degree of IgE sensitization and the level of allergen exposure (3). Furthermore, the much better fit in the never-smoker sample indicates that the effect of smoking is also highly variable, but this factor could possibly be improved by using an objective marker of the degree of cigarette smoke exposure. However, these limitations do not rule out the benefit of adjusting for the more easily predictive effect of, for example, age, height, and gender, on expected normal FeNO values (7, 11) since these factors have a similar impact on FeNO in healthy subjects and patients with asthma (2). We have previously shown in a systematic review that FeNO may be higher in children with atopy as well as in children with allergic rhinitis, whereas in adults, an increase has only been observed in allergic rhinitis, with FeNO being similar in atopic and healthy individuals (23). However, there is no reliable information on rhinitis in NHANES subjects (8).

In conclusion, we suggest a novel model for the prediction of reference FeNO values that can contribute to the interpretation of FeNO in clinical practice, adapted to each individual subject, and adjusted for explanatory variables. This is an approach similar to the current paradigm of reference values in spirometry (31). The model should be further validated in large samples of subjects with an objective measurement of atopy and a medical diagnosis of asthma and rhinitis.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

T.J., J.F., and K.A. conceived and designed research; T.J. and R.A. performed experiments; T.J. and R.A. analyzed data; T.J., R.A., A.M., C.J., J.F., and K.A. interpreted results of experiments; T.J. prepared figures; T.J., A.M., C.J., and K.A. drafted manuscript; T.J., A.M., C.J., J.F., and K.A. edited and revised manuscript; T.J., R.A., A.M., C.J., J.F., and K.A. approved final version of manuscript.

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