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Reference Ranges and Determinant Factors for Fractional Exhaled Nitric Oxide in a Healthy Saudi Adult Population

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Statistical Analysis C
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Literature Search F
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Background: Fractional exhaled nitric oxide (FENO) has emerged as a promising marker in respiratory research. The aim of this study was to determine the reference range values of FENO for healthy Saudi adults and the factors associated with FENO levels.

Material/Methods: This cross-sectional study was conducted at the Department of Physiology, King Saud University, Riyadh, Saudi Arabia, from January 2016 to August 2017. A total of 429 healthy Saudi adults were initially recruited. The final selection included 412 participants, consisting of 307 men and 105 women. FENO measurements were performed according to the current recommendations of the American Thoracic Society.

Results: We observed that the FENO levels of women were significantly lower than those of men (18.6 vs. 21.3, $P=0.009$). In women, the measured FENO ranged from 5.7 ppb to 42 ppb, and in men from 5.0 ppb to 55.0 ppb. The mean FENO level in the entire study population was 20.6, with a range of 5.0 ppb to 55.0 ppb. The difference became non-significant when we calculated the FENO after adjusting for body surface area by different percentile distributions. Multiple linear regression analysis showed that body surface area and weight were significant predictors of FENO levels.

Conclusions: In this study, FENO levels were significantly affected by demographic variables. Therefore, it is important to consider the factors influencing FENO values to make a valid clinical interpretation.

MeSH Keywords: **Body Mass Index • Body Surface Area • Gender Identity • Nitric Oxide • Reference Values**

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Background

Fractional exhaled nitric oxide (FENO) is a molecule involved in the regulation of bronchial and vascular tone and inflammation of the bronchial mucosa [1,2]. FENO is a non-invasive marker of inflammation driven by helper T cells in airways, which are mediated by interleukin-4 and interleukin-13 [3,4]. It is currently one of the diagnostic tests used in clinical practice to determine the diagnosis and prognosis of many airway diseases [5,6]. The role of FENO is also described in the process of neurotransmission. Many diseases of the respiratory tract, such as bronchial asthma [7] and chronic obstructive pulmonary disease [8], are related to an increased concentration of FENO [9]. The use of anti-inflammatory drugs has been shown to reduce FENO levels [10,11]. In addition, FENO values are affected by age, race, sex, anthropometric measurements, atopy, and smoking habits [12]. These factors make the interpretation of diagnostic tests difficult. The diagnostic test can be interpreted in many ways, and the American Thoracic Society has suggested that using an absolute value for FENO is not recommended, which is also the case for lung function tests. Furthermore, fixed cutoff values for FENO are not specified in the society's guidelines [13]. Similarly, reports in the literature differ regarding the normal FENO value among different populations, and there are different results reported for FENO values in various populations and in relation to confounding factors like smoking, which lowers FENO values [14,15].

FENO values have been very useful in assessing the diagnosis and prognosis of asthma [16]. However, its use seems to be limited in other chronic respiratory diseases, like chronic obstructive pulmonary disease and bronchiectasis [17]. We previously published the reference range values for the healthy male adult Saudi population [18]. However, differences in FENO levels due to sex and reference ranges for the healthy adult female population and their relation to age, height, weight, and BMI have not been evaluated in detail. Therefore, this study aimed to determine the reference range values of FENO for healthy Saudi adults and to determine the factors associated with FENO levels.

Material and Methods

This cross-sectional study was conducted at the Department of Medicine and Physiology, King Saud University Medical City, King Saud University in Riyadh, Saudi Arabia, from Jan 2016 to Aug 2017. The study was approved by the Ethics Review Board of the College of Medicine, King Saud University. Written informed consent was obtained from all participants and a brief questionnaire was completed for all. Healthy adult participants (minimum age of 18 years) with normal spirometry recordings were included in the study. A special clinic was set up in the Department of Physiology of the King Saud University.

Participants were informed about the study through poster advertisements and the internet. Because the main focus of the study was to determine reference ranges for healthy Saudi adults and to determine the confounding factors for FENO values, we excluded individuals with (1) age under 18 years; (2) history of smoking; (3) airway obstruction with forced expiratory volume in 1 s (FEV1) <80% of the predicted normal value; (4) any clinical manifestation of allergy from atopy or history of allergic diseases such as asthma, allergic rhinitis, urticaria, and allergic dermatitis; (5) infection within the previous 3 weeks; (6) associated lung pathologies including chronic obstructive pulmonary disease, bronchiectasis, and chronic bronchitis; and (7) corticosteroid usage.

A total of 429 participants were initially recruited and 412 (307 men and 105 women) met the inclusion and exclusion criteria and were selected to participate in the study. We excluded 17 participants from the study either because of exclusion criteria or because they were unable to perform the test procedures in accordance with the American Thoracic Society guidelines.

Measurement of fractional exhaled nitric oxide

FENO measurements were performed and calculated as per the current American Thoracic Society guidelines (6) using an EVA Nox 4000 chemiluminescence analyzer (Seres, France), which has the sensitivity to measure FENO in 1 part per billion (ppb) of exhaled air. All participants were asked to rest and avoid eating, drinking, and any strenuous exercise for about 3 h before the measurement of FENO levels. All measurements were performed between 9–11 a.m., to minimize possible effects of the circadian rhythm. In addition, diet history was obtained to avoid any alterations in FENO levels due to nitrate-containing foods. To calculate the slow vital capacity, participants were asked to fill their lungs from the residual lung volume to total lung capacity and then perform slow expiration. They were asked to keep a constant expiratory flow rate of about 0.05 L/s, and to maintain it for at least 15 s, exhaling into a Teflon cylinder connected to Teflon tubing. An expiratory resistance of 10 cm to 20 cm was applied to exclude any nasal contamination. The resistance was detected by a Samba sensor 3200 (Samba Sensors AB, Vastera Frolunda, Sweden), a special sensor for making flow rate recordings. The expiratory flow rate for each participant was measured by a computerized data acquiring software system (BIOPAC MP-100, BIOPAC Systems Inc, Goleta, GA, USA). The plateau levels of FENO against time were determined, and FENO levels were calculated as ppb. FENO concentrations were graphed and averaged from 5 to 15 s after the start of expiration. The average values of 3 successive samples taken with at least a 1-min interval in between were calculated. For standardization, the EVA Nox machine was calibrated daily before each experiment, and the variation between tests was kept within the range of 10%.

Table 1. Clinical and demographic characteristics and FENO levels of all participants (n=412).

Variables	Mean±SD	Min–Max
Age	36.9±15.2	18.0–72.0
Height	167.9±8.5	147.0–187.0
Body surface area	2.8±0.3	2.0–3.5
Weight (kg)	76.7±17.5	18.3–140.0
Obesity degree (%)	127.6±26.8	74.0–268.0
BMI	27.2±5.7	15.6–55.4
Fitness Score	70.2±7.5	29.0–95.0
FENO (ppb)	20.6±9.1	5.0–55.0

BSA – body surface area; BMI – body mass index; FENO – fractional exhaled nitric oxide; ppb – parts per billion.

Ventilatory functions

All spirometric recordings including FEV1, peak expiratory flow (PEF), forced vital capacity (FVC), and FEV1/FVC ratio were performed using an electronic spirometer (Vitalograph, Ireland) after FENO recordings. All measurements were taken 3 times and with participants in a seated position.

Statistical analysis

Data were entered in Microsoft excel and data analyses were performed using SPSS for windows version 20.0. Prior to the final analysis, data were screened for normality assumption, homogeneity of variance, and presence of extreme scores. The homogeneity of the variance test and test of normality were done using the Shapiro-Wilk test. If these results were significant, defined as a *P* value less than 0.05, then the non-parametric Mann-Whitney U test was used to compare the differences by sex; otherwise, an independent *t* test was used

for normally distributed data. For multiple group comparisons, one-way ANOVA was used, along with a post hoc Bonferroni test. Spearman and Pearson correlations were performed as necessary. Multiple linear regression analysis was performed with FENO as the dependent variable and age, sex, body surface area, height, weight, and body mass index (BMI) as independent variables. Frequency distributions at different range values were computed for FENO. All tests were 2-tailed with a *P* value of ≤0.05 considered as statistically significant.

Results

In this study, we determined the reference range values of FENO and their distribution in adult female Saudi participants. All participants were healthy and nonsmokers. Table 1 shows the clinical characteristics and FENO levels of all participants. The mean FENO level of the entire study population was 20.6±9.1 ppb, ranging from 5.0 ppb to 55.0 ppb. Table 2 shows the comparison of clinical characteristics and FENO levels between male and female participants. We observed that women had significantly lower FENO levels compared to those of men (18.6 vs. 21.3; *P*=0.009). In women, FENO levels ranged from 5.7 to 42.0 ppb, and in men they ranged from 5.0 to 55.0 ppb. However, after adjusting for body surface area, the difference was non-significant (7.3±4.4 ppb in women vs. 7.4±3.4 ppb in men; *P*=0.784). Figure 1 shows the data distribution histogram of different ranges of FENO levels with the percentage of distribution in each group. The mean age of the entire study population was 36.9±15.2 years (range, 18 to 72 years); height, 171.79±8.3 cm; weight, 80.17±18.38 kg; BMI, 27.23±6.64; and FEV1/FVC, 85% (81–92%) (Table 1). Figure 2 shows the distribution of FENO levels according to different categories of BMI. Participants who were overweight had slightly higher FENO levels than participants who were normal weight, and participants with third degree obesity had slightly lower

Table 2. Differences by sex in demographic characteristics, and FENO levels in all participants.

Variables	Women (n=105)		Men (n=307)		<i>P</i> value
	Mean±SD	Min–Max	Mean±SD	Min–Max	
Age (years)	30.7±9.9	14.0–53.0	39.1±16.1	13.0–72.0	0.001
Height (cm)	159.4±5.5	148.0–173.0	170.8±7.3	147.0–187.0	0.001
BSA	2.5±0.2	2.2–3.0	2.9±0.3	2.3–3.5	0.001
Weight (kg)	66.2±15.1	36.1–140.0	80.3±16.8	18.3–135.7	0.025
Obesity Degree (%)	126.8±30.6	78.0–268.0	127.9±25.5	74.0–211.0	0.001
BMI	26.1±6.2	15.6–55.4	27.6±5.4	15.6–45.9	0.036
FENO (ppb)	18.6±9.3	5.7–42.0	21.3±8.9	5.0–55.0	0.009
FENO/BSA	7.3±4.4	2.2–17.7	7.4±3.4	1.6–31.9	0.784

BSA – body surface area; BMI – body mass index; FENO – fractional exhaled nitric oxide; ppb, parts per billion.

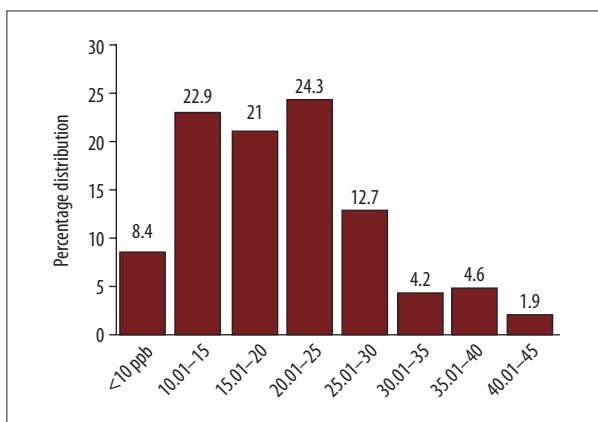


Figure 1. Ranges of fractional exhaled nitric oxide (FENO) values at different levels of distribution in females.

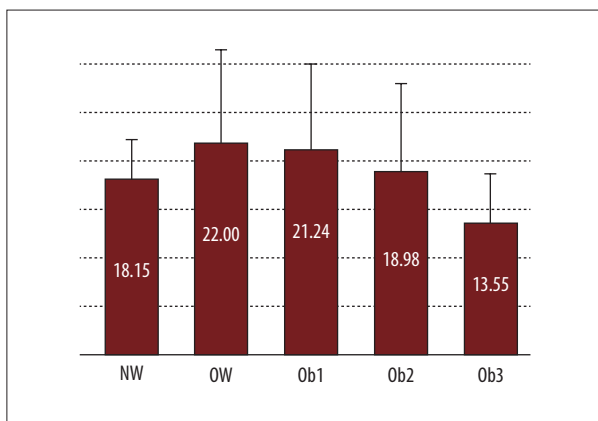


Figure 2. Distribution of fractional exhaled nitric oxide (FENO) according to different categories of body mass index (BMI). NW – normal weight; OW – overweight; Ob1, Ob2, and Ob3 – Obesity class 1, 2, and 3.

FENO levels than participants who were overweight (overall ANOVA P value=0.007). Post hoc analysis revealed that the differences between the groups were non-significant. The relationship between FENO and body surface area showed a significant positive correlation for all participants ($r=0.188$; $P=0.001$). The relationship was significant for men ($r=0.142$; $P=0.013$), while for women it was non-significant ($r=0.118$; $P=0.229$) (Figure 3). When adjusted for body surface area, the correlation became non-significant for all ($r=-0.019$; $P=0.126$), men ($r=-0.150$; $P=0.126$), and women ($r=-0.153$; $P=0.119$) (Figure 4). The relationship between FENO and age was also non-significant ($r=-0.052$; $P=0.029$) (Figure 5). Multiple linear regression analysis showed that body surface area and weight were significant predictors of FENO levels (Table 3).

Figure 6 shows the comparison of FENO levels between male and female participants according to different percentiles of body surface area, with mean values and 95% confidence intervals. It was observed that at <25%, 25–50%, 50–75%, and

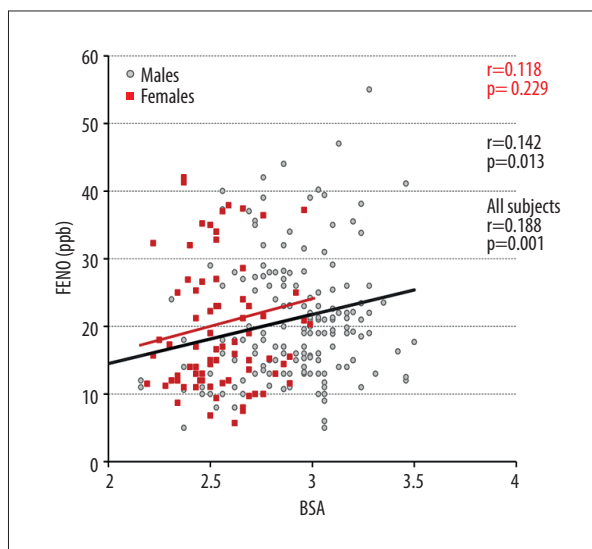


Figure 3. The relationship between fractional exhaled nitric oxide (FENO) levels and body surface area (BSA) in all participants, men and women.

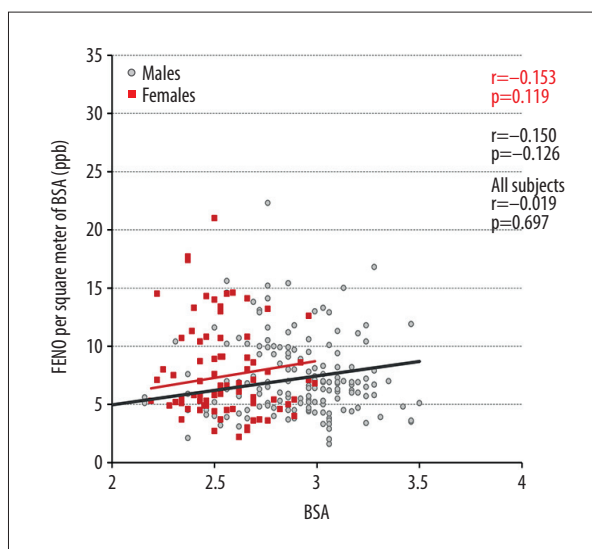


Figure 4. The relationship between adjusted fractional exhaled nitric oxide (FENO) levels and body surface area (BSA) in all participants, men and women.

>75% of body surface area the difference was non-significant with P values of $P=0.9645$, $P=0.0572$, $P=0.4451$, and $P=0.0631$, respectively. This revealed that our results showed significantly lower FENO levels in women compared to those in men. However, adjusting for body surface area made the difference non-significant in the different percentile distributions.

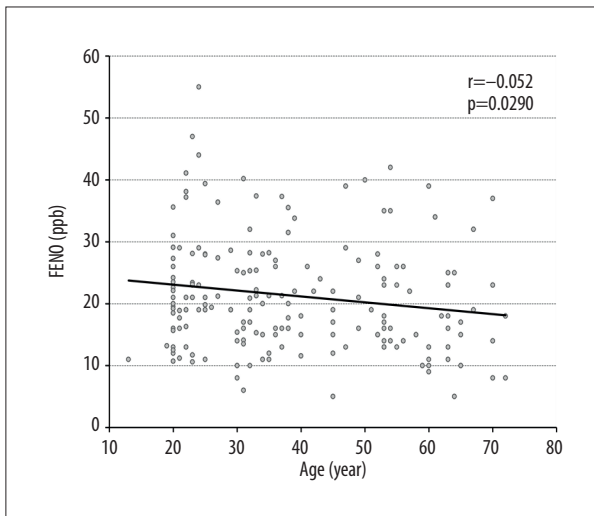


Figure 5. The relationship between fractional exhaled nitric oxide (FENO) levels and age in years in all participants.

Discussion

The primary objectives of this study were to determine differences in FENO levels according to sex, and to develop reference ranges of FENO levels in healthy female Saudi participants, measured in accordance with current recommended standards. Our results showed that women had significantly lower FENO levels than did men. However, when adjustments were made for body surface area, the difference in FENO levels between female and male participants was non-significant. To the best of our knowledge, the current study is the first to analyze body surface area adjustments for FENO. In our previous study, FENO levels ranged between 7.66 ppb and 46.6 ppb (mean 22.79 ± 8.13 ppb) in adult men, levels correlated negatively with body weight ($r = 0.388$, $P = 0.001$) and BMI ($r = 0.238$, $P = 0.009$), and no correlations were observed among FENO, FEV1/FVC ratio, age, and height [18]. The present study showed similar mean values for men (21.3 ± 8.9). In a study by Toren et al., the population median FENO value was 16.5 ppb,

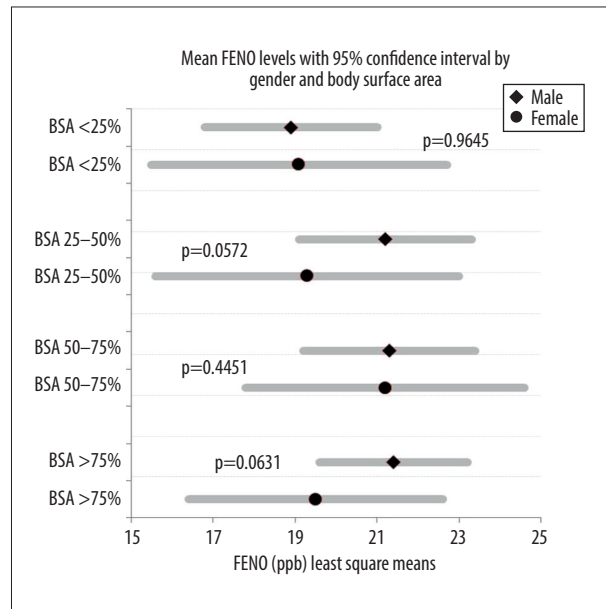


Figure 6. Comparison of mean fractional exhaled nitric oxide (FENO) level distribution between men and women according to different percentiles of body surface area (BSA).

with 7.2 ppb and 39.0 ppb as the 5th and 95th percentile values, respectively. FENO levels were significantly ($P < 0.0001$) higher in men than in women in their study [19]. However, they did not adjust the values for body surface area as we have done in our current study.

Low levels of FENO in women have been previously reported, but the findings of our current study are novel because when we adjusted for the total body surface area, the absolute FENO values of women were not significantly different from those of men. This effect can be partly explained by differences in height, because the differences between male and female participants were not consistent after an adjustment was made for height. This probably points to a difference in NO production

Table 3. Multiple linear regression analysis with FENO as dependent variable with demographic factors as independent variables.

	Beta standardized coefficients	t	P value	95.0% Confidence interval for B lower bound – upper bound
Age	-.084	-1.542	0.124	-.114–.014
Sex	-.088	-1.341	0.181	-4.544–.859
BSA	.164	3.368	0.001	2.183–8.303
Height	2.364	1.407	0.160	-.998–6.025
Weight	-.371	-2.290	0.023	-.358 – -.027
BMI	.207	1.359	0.175	-.148–.812

BSA – body surface area; BMI – body mass index.

between the sexes [20], a relationship that has been attributed to the smaller airway size in females, as airway surface is one source of NO production [21]. The current findings are also consistent with the strong relationship between height with FENO that was observed in children [21]. However, with adjustments for body surface area, the differences related to male or female sex were insignificant, although the absolute levels were significantly lower in female participants. This suggests that not only the airway surface area, but also the whole body surface affects FENO production.

Jo et al. reported that the significant factors for FENO are weight, height, BMI, and atopy, while age is not a significant determinant. In their multiple linear regression analysis, sex and weight were found to have significant associations with FENO [14]. Their observations support our results, but with some differences. In our current study, weight and body surface area were the only independent factors associated with FENO levels. The effect of collinearity could have made the predictive value of other factors in our study non-significant. The different results between the studies could be also be attributable to our analyzing for the effect of body surface area, whereas the other researchers did not [14].

NO formation is already detected at birth [22,23] and is important in humans, which are under strict biological control. NO formation is a complex and energy consuming process whereby NO is produced by inducible NO synthase in the airway epithelium. This indicates that the total surface area of the airway epithelium/mucosa is important in the detection of FENO levels, and was theoretically shown to negatively correlate with the anatomic dead space volume in healthy children [24]. Therefore, it is assumed that anthropometric factors are important in evaluating FENO levels, as was previously observed for lung function parameters [19]. This was also demonstrated in linear regression models of FENO levels in many studies which had results similar to our current study [25,26].

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Conclusions

Our results revealed that FENO levels are significantly affected by demographic variables. Women have lower FENO values than do men. However, after adjusting for body surface area, the difference becomes non-significant. Therefore, it is important to consider the factors influencing FENO values to make valid clinical interpretations. Future studies should consider racial and genetic differences. Larger sample sizes are needed to develop standard reference equations based on predetermined physiological models for the evaluation of normal FENO.

Conflict of interest

None.

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