

Reference Ranges for Exhaled Nitric Oxide Fraction in Healthy Japanese Adult Population

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ABSTRACT

Background: The measurement of the exhaled nitric oxide fraction (F_{ENO}) is proposed as a useful marker of airway inflammation. In healthy adults, there have been a few studies of the reference ranges for F_{ENO} in Caucasians. A community study in other regions may reveal any possible ethnic differences in the F_{ENO} levels.

Methods: A total of 240 healthy adults aged between 18 to 74 years were recruited from four medical centers in Japan. Current smokers and subjects having a history of atopic disease were not included. F_{ENO} was measured using an online electrochemical nitric oxide analyzer according to the current guidelines. The reference ranges for F_{ENO} were estimated using two different statistical methods recommended by International Federation of Clinical Chemistry and Laboratory Medicine.

Results: The mean F_{ENO} was 16.9 ppb (parts per billion) with a 95% prediction interval (2.5 to 97.5 percentiles) of 6.5 to 35.0 ppb in healthy Japanese adults. Normality assumptions were met for the logarithm-transformed F_{ENO}. The geometric mean F_{ENO} was 15.4 ppb with a mean $\pm$ two standard deviations of 6.5 to 36.8 ppb. Age, gender, height, and past smoking history were not associated with the F_{ENO} levels.

Conclusions: The reference ranges for F_{ENO} in healthy Japanese adults were similar to those of Caucasians. It seems reasonable that the upper limit of F_{ENO} for healthy adults should be set at approximately 36.0 ppb irrespective of ethnic differences.

KEY WORDS

airway inflammation, asthma, atopy, ethnic difference, smoking

ABBREVIATIONS

BMI, Body mass index; F_{ENO}, Exhaled nitric oxide fraction; ppb, Parts per billion.

INTRODUCTION

The measurement of the exhaled nitric oxide fraction (F_{ENO}) has been proposed as a useful marker of airway inflammation.¹ F_{ENO} levels are elevated in inflammatory lung diseases, such as asthma.^{1,3} Establishing reference ranges in healthy subjects would be useful for the interpretation of F_{ENO} measurements. Although the measurement procedures have been standardized, the normal upper limits of F_{ENO} levels have not been specified.¹

Previous studies have demonstrated that there are

several determinants of F_{ENO}, such as age, gender, atopy, smoking status, and diet.^{1,4-17} It has been reported that the F_{ENO} levels in Asian children are significantly higher than those in Caucasian children.¹⁵⁻¹⁷ However, in healthy adults, there have been a few studies of the reference ranges for F_{ENO} in Caucasians.⁴⁻⁶ A community study in other regions may reveal any possible ethnic differences in the F_{ENO} levels.

In the present study, the reference ranges for F_{ENO} in healthy Japanese adults were estimated using two different statistical methods recommended

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Table 1 Demographics of study subjects

Number	240
Gender (Female/Male)	131/109
Smoking status (Nonsmoker/Ex-smoker)	197/43
Age (years)	39.4 ± 13.6
Height (m)	1.63 ± 0.08
Body weight (kg)	59.6 ± 11.5
Body mass index (kg/m ²)	22.2 ± 3.1

Values are Mean ± SD.

by International Federation of Clinical Chemistry and Laboratory Medicine,¹⁸ and the results were compared with those of Caucasians. Furthermore, factors influencing the FENO levels were also investigated.

METHODS

STUDY SUBJECTS

A general population sample of people aged 18 years or older was randomly selected from the population register in Wakayama, Fukuoka, Kanagawa, and Tokyo, Japan. All participants were interviewed by physicians and a total of 240 healthy adults were recruited. The study was approved by the local ethics committee and informed consent was obtained from each subject. To avoid the influence of the pollen season, the enrollment was performed from May to July 2009. The study subjects had neither history of atopic rhinitis, atopic dermatitis, food allergy, nor history of asthma, or other lung diseases. We did not include those subjects who reported having symptoms of either asthma or rhinitis based on guidelines.^{19,20} Subjects were not included if they were current smokers or ex-smokers with more than 20 pack-years, had had an airway infection or were taking any form of corticosteroids in the 4 weeks preceding the study. Baseline demographics of the subjects are presented in Table 1.

STUDY DESIGN

This was a multi-center cross-sectional study. The subjects attended the outpatient clinic on one occasion for physical examination and FENO measurements.

FENO MEASUREMENTS

FENO was measured by an online electrochemical nitric oxide analyzer (NIOX MINO; Aerocrine AB, Solna, Sweden). This nitric oxide analyzer has been approved by the U.S. Food and Drug Administration for clinical use. Measurements of FENO were performed asking the subjects to empty their lungs and then to inhale to total lung capacity through the mouthpiece and finally exhale into device at a constant flow rate of 50 mL/s; the software within the device automatically checks that the breathing manoeuvre is performed according to American Thoracic So-

Table 2 Geometric mean and reference ranges for logarithm-transformed FENO

Group	Geometric mean (SD)	90% confidence interval	Mean ± 2 SD
All	15.44 (1.54)	14.75, 16.17	6.49, 36.76
Female	14.64 (1.59)	13.69, 15.66	5.79, 37.04
Male	16.46 (1.47)	15.48, 17.51	7.60, 35.68
Nonsmoker	15.34 (1.56)	14.55, 16.17	6.27, 37.57
Ex-smoker	15.91 (1.44)	14.49, 17.48	7.67, 35.02

Definition abbreviation: SD, standard deviation.

ciety/European Respiratory Society guidelines.¹ The calibration of the analyzer is automatically performed by the software. The sensor on the device was changed periodically according to the manufacture's guidance. Repeated exhalations were performed to obtain two acceptable measurements that agreed within 10% deviation, and the average of these two values was registered. All subjects were fasted for one hour before the FENO measurements.

STATISTICAL ANALYSIS

The reference ranges for FENO were estimated using two different statistical methods recommended by International Federation of Clinical Chemistry and Laboratory Medicine: 1) 95% prediction interval (2.5 to 97.5 percentiles) of FENO values; 2) Since normality assumptions were met for the logarithm of FENO, the logarithm-transformed value was used to calculate the mean ± two standard deviations (SD) and then back-transformed values were estimated.¹⁸

The factors influencing the FENO values were examined in a regression tree-based model with the logarithm-transformed FENO as the response variable in relation to the different explanatory variables, age, gender, height, and past smoking history. Comparisons of mean FENO values between the groups (gender and smoking status) were performed by unpaired *t* tests. Pearson's correlation coefficients were calculated to determine the correlation between the FENO values and continuous data (age and height). All data were expressed as mean ± SD and significance was defined as a *p* value of less than 0.05.

RESULTS

The reproducibility of FENO measurements was expressed as the limit of agreement (intraclass correlation coefficient = 0.97). The mean FENO was 16.9 ppb (parts per billion) with a 95% prediction interval of 6.5 to 35.0 ppb. Normality assumptions were met for the logarithm-transformed FENO (Kolmogorov-Smirnov test, *p* = 0.43) and back-transformed values were used in the subsequent analysis. The geometric mean FENO for all subjects was 15.4 ppb with a mean ± 2 SD of 6.5 to 36.8 ppb in healthy Japanese adults (Table 2).

The mean FENO was 1.13 times higher in males,

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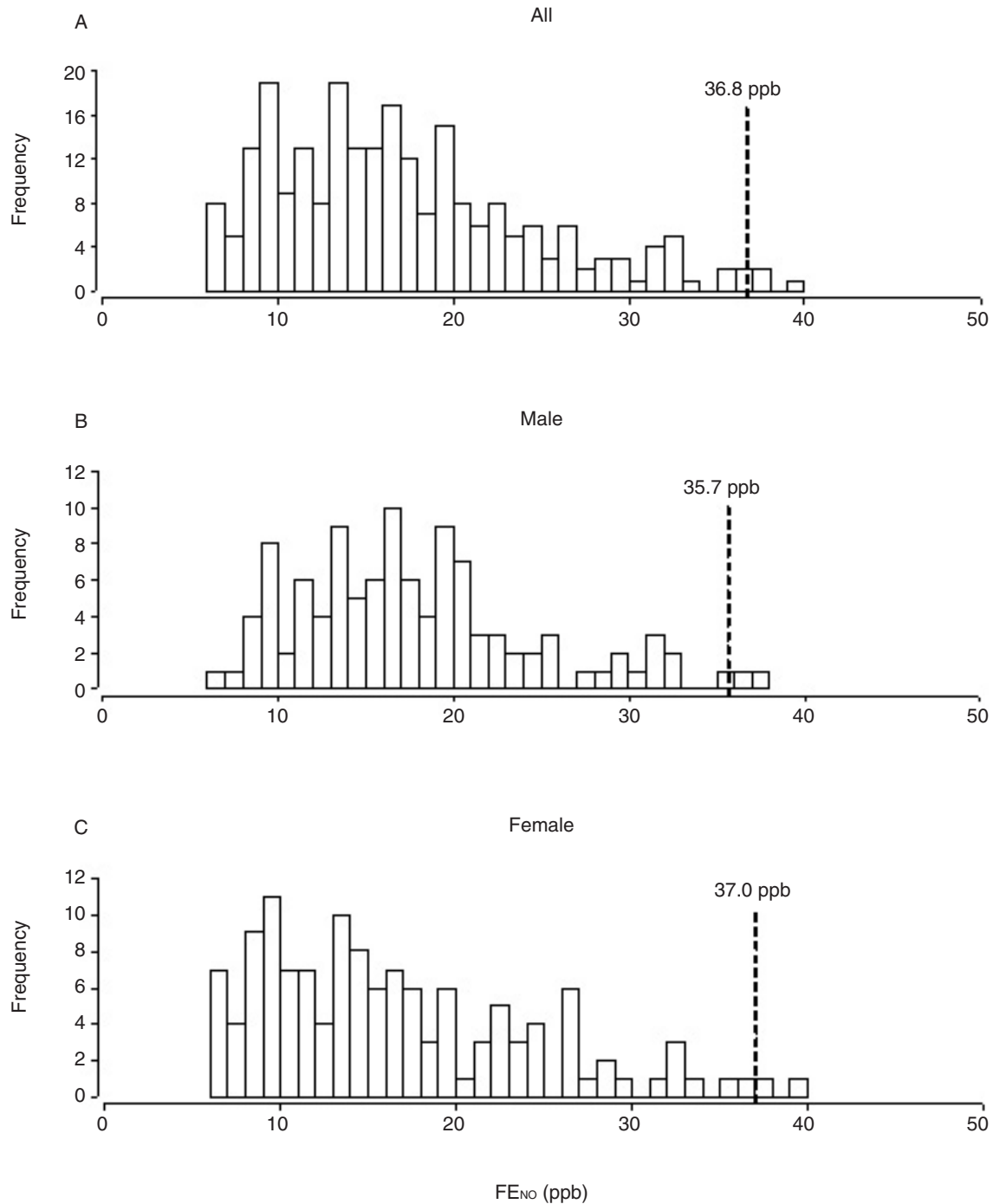


Fig. 1 The distribution of the FE_{NO} levels in all subjects (A), males (B), and females (C). The dotted lines correspond to the upper limits of the reference range for the logarithm-transformed FE_{NO} (mean + 2 standard deviation) in each group.

16.5 ppb, compared to the 14.6 ppb in females ($p < 0.05$), and that was 1.04 times higher in ex-smokers, 15.9 ppb, compared to the 15.3 ppb in nonsmokers ($p = 0.62$). However, the mean $\pm$ 2 SDs of FE_{NO} values were similar in each group although it was not statistically analyzed (Table 2). The distribution of the FE_{NO} levels in all subjects, males, and females, and

the upper limits of FE_{NO} in each group are shown in Figure 1. In a linear regression analysis, no correlations were found between FE_{NO} and the values of age ($r = 0.12$, $p = 0.07$) or height ($r = -0.02$, $p = 0.82$). The regression tree analysis showed that age, gender, height, and past smoking history were not significant factors influencing the FE_{NO} levels.

DISCUSSION

The present study is one of the largest that investigated the FENO levels in a community sample of adults. We have shown that the reference range for FENO was approximately 6.5 to 36.0 ppb in healthy Japanese adults.

For healthy Caucasian adults, a few reports have shown the reference ranges for FENO measurements according to the current guidelines.^{4,6,8} Travers *et al.* recruited 193 normal subjects categorized as follows: no physician diagnosis of lung disease, no symptoms of lung disease, no inhaled medication, and no allergic rhinitis.⁵ The criteria were similar to ours although 19 current smokers were included in the study population. The geometric mean FENO was 17.9 ppb with a 90% confidence interval of 7.8 to 41.1 ppb.⁵ In another study comprising 30 healthy non-atopic subjects, the mean FENO was 16.3 ppb with SD of 8.4 ppb in adults.⁷ On the basis of this report, a recent review suggested 33.1 ppb (mean + 2 SD) as the upper limit of FENO for adults.⁸ Olin *et al.* recruited healthy adults from the general population, and 1,131 never smokers, including 845 non-atopic and 286 atopic subjects, were selected for the study.⁶ Subjects with physician-diagnosed asthma or asthma symptoms, and those using inhaled steroids were excluded. Using a reference equation based on multiple regression modeling, they proposed upper limits of FENO ranging from 24.0 ppb to 54.0 ppb depending on age and height.⁶ This upper limit of FENO seems definitely higher than those reported in previous studies and ours. In their study, the subjects having symptoms of rhinitis were not excluded, and this population comprised about 45% of the study subjects. The high prevalence of subjects with nasal symptoms may explain the increased FENO levels. In the present study, we estimated the reference ranges for FENO using two different statistical methods recommended by the guidelines,¹⁸ and this study demonstrated approximately 36.0 ppb as a reasonable upper limit of FENO for healthy Japanese adults, a value that is within previously suggested limits for Caucasians.

It has been shown that the mean FENO levels in healthy Asian children are significantly higher than those in healthy Caucasian children.¹⁵⁻¹⁷ Several factors, such as dietary differences, environmental differences, and genetic variation have been proposed to explain the ethnic differences.¹⁵⁻¹⁷ However, these studies included less than 70 subjects who were ethnic minorities, and the reference ranges for FENO in each ethnic group were not estimated. In addition, FENO was measured according to the current guidelines in only one of these studies.¹⁷ Further study with large samples using standardized methods will be necessary to clarify ethnic differences in the FENO levels of children.

The normal range for FENO is influenced by patient

factors. Previous studies have shown that there are several determinants of FENO levels, such as age, gender, height, atopy, smoking, and diet.^{1,4-17} The finding of a higher FENO in subjects with atopy has been reported previously,^{5,9-11} while chronically reduced levels of FENO have been demonstrated in current smokers.^{5,9,12} Therefore, current smokers and subjects having a history of atopic disease were not included in this study. Furthermore, the FENO level has been found to be elevated after the intake of a nitrate-rich meal.^{13,14} Thus, the subjects were fasted for one hour before the FENO measurements. In the present study, the mean FENO level for males was significantly higher than that for females, which is consistent with previous reports.^{4,5,7,15} However, the upper limits of FENO were similar in each gender group as shown in Figure 1. In addition, age, gender, height, and past smoking history were not significant predictors for FENO. Although the association between FENO and age, gender, height is still controversial in adults,^{1,4-9} the present results suggested that these demographics are not critical factors in establishing the normal range for FENO.

In the present study, subjects were recruited based on interview. Although the subjects with a history of atopic disease were carefully excluded according to the guidelines,^{19,20} we made no attempt to validate information by skin prick testing. However, a detailed interview is a basic approach to investigate individual demographics, and it will not always be practical for clinicians to perform skin prick testing before FENO measurements. In clinical practice, the proposed reference data would be useful for interpretation of the FENO measurements as a reasonably approximate value. Additionally, the reference ranges for FENO in Caucasians adults were analyzed by different measurement system, chemiluminescence analyzer.⁵⁻⁷ However, it has been shown that FENO values measured by the electrochemical analyzer are reproducible, reliable, and in agreement with the chemiluminescence analyzer.²¹⁻²⁴

In summary, the reference ranges for FENO in healthy Japanese adults were similar to those of Caucasians. It seems reasonable that the upper limits of FENO for healthy adults should be set at approximately 36.0 ppb irrespective of ethnic differences.

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