Determinants of fractional exhaled nitric oxide in healthy men and women from the European Community Respiratory Health Survey III

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Abstract

Introduction: The fractional exhaled nitric oxide (\(F_{E}\)NO) is a marker for type 2 inflammation used in diagnostics and management of asthma. In order to use \(F_{E}\)NO as a reliable biomarker, it is important to investigate factors that influence \(F_{E}\)NO in healthy individuals. Men have higher levels of \(F_{E}\)NO than women, but it is unclear whether determinants of \(F_{E}\)NO differ by sex.

Objective: To identify determinants of \(F_{E}\)NO in men and women without lung diseases.

Method: Fractional exhaled nitric oxide was validly measured in 3881 healthy subjects that had answered the main questionnaire of the European Community Respiratory Health Survey III without airways or lung disease.

Results: Exhaled NO levels were 21.3% higher in men compared with women \(P < 0.001\). Being in the upper age quartile (60.3–67.6 years), men had 19.2 ppb (95% CI: 18.3, 20.2) higher \(F_{E}\)NO than subjects in the lowest age quartile (39.7–48.3 years) \(P = 0.02\). Women in the two highest age quartiles (54.6–60.2 and 60.3–67.6 years) had 15.4 ppb (14.7, 16.2), \(P = 0.03\) and 16.4 ppb (15.6, 17.1), \(P < 0.001\) higher \(F_{E}\)NO, compared with the lowest age quartile.

Height was related to 8% higher \(F_{E}\)NO level in men (\(P < 0.001\)) and 5% higher \(F_{E}\)NO levels in women (\(P = 0.008\)). Men who smoked had 37% lower \(F_{E}\)NO levels and women had 30% lower levels compared with never-smokers (\(P < 0.001\) for both). Men and women sensitized to both grass and perennial allergens had higher \(F_{E}\)NO levels compared with non-sensitized subjects 26% and 29%, \(P < 0.001\) for both.

Conclusion and Clinical Relevance: Fractional exhaled nitric oxide levels were higher in men than women. Similar effects of current smoking, height, and IgE sensitization were found in both sexes. \(F_{E}\)NO started increasing at lower age in women than in men, suggesting that interpretation of \(F_{E}\)NO levels in adults aged over 50 years should take into account age and sex.

Keywords
fractional exhaled nitric oxide, healthy population, IgE sensitization, smoking
1 | INTRODUCTION

Nitric oxide (NO) serves many functions throughout the body. It is produced by the epithelium as part of the immune defence against pathogens and is involved in neurotransmission in the peripheral and central nervous systems, as well as the regulation of vascular and bronchial tone.

Exhaled NO reflects mainly the respiratory epithelium production of NO, resulting from activation of inducible NO synthase (iNOS), which is controlled by signal transducer and activator of transcription (STAT)-1 under the influence of homeostatic interferon-γ. The concentrations are generally low in healthy individuals. However, high concentrations of exhaled NO are seen in chronic inflammatory diseases, such as asthma, mainly due to type 2 inflammation resulting in increased activation of iNOS. Airway infections, especially rhinovirus, and allergic rhinitis are also related to higher levels of exhaled NO.

The measurement of fractional exhaled NO (FENo) is a useful, non-invasive method to assist with the diagnosis of asthma and monitor treatment effects. In recent years, FENo has been used as a marker for eosinophilic airway inflammation and asthma, and to identify steroid responsiveness in individuals with chronic respiratory symptoms caused by airway inflammation.

For FENo to be reliable as a biomarker, it is important to know factors that influence FENo values. Currently, it is known that FENo values are influenced by age, gender, height, atopy, smoking, respiratory infections, environmental factors, physical activity, and ethnicity.

Females have consistently been reported to have lower FENo levels than men with about 25% lower levels. Some of the explanation might reside in differences in height, another known determinant of FENo, but other differences appear to be exist with regard to gender. Moreover, the effect of different known determinants of FENo has not been studied with regard to gender. Specifically, the relation with age appears to be different with regard to gender, as a recent publication suggests that after a period in early adulthood with no relation between age and FENo, an increase in FENo with age is found at age around 45 in women and 59 years in men.

In this study, we aimed to describe determinants of FENo in men and women, with special emphasis on gender differences, in subjects without lung disease (asthma, chronic obstructive lung function, and emphysema) in the third European Community Respiratory Health Survey (ECRHS III).

2 | METHODS

2.1 | Study sample

This is a cross-sectional analysis based on the third follow-up of ECRHS (ECRHS III), performed between the years 2010-2013, using data from 25 centres across 11 European countries and Australia.

Briefly, ECRHS is an international multi-centre population-based study of asthma and allergy, which was first performed in the early 1990s. The subjects, age 20-44 years, were first randomly selected to complete a short postal questionnaire about asthma symptoms and attacks in the preceding 12 months, current use of asthma medication, and the presence of nasal allergies including hayfever. Both a random sample and a symptomatic sample of responders were invited to attend further examinations at their study centre. Current analysis is based on the random population sample. Follow-up studies were performed in 2000-2002 (ECRHS II) and 2010-2013 (ECRHS III). Further details about ECRHS have been published elsewhere and can also be found on the homepage: www.ecrhs.org.

Of 5483 participants in ECRHS III, 1004 were excluded due to current asthma and/or asthma symptoms in the last 12 months, 83 due to self-reported physician-diagnosed chronic obstructive pulmonary disease and emphysema, and 176 due to use of inhaled medicines in the last 12 months. Further, 339 subjects with respiratory symptoms at ECRHS I (symptomatic sample) were also excluded. Thus, the final study population included 3881 participants, aged 39-77 years (men: 40-73 years), who underwent FENo and other clinical tests, and responded to questions about respiratory symptoms and smoking habits.

2.2 | Questionnaires and measurements

Participants had to be free from respiratory infections the 2 weeks preceding the clinical examination. An interviewer-led questionnaire contained questions on respiratory symptoms, self-reported asthma, chronic obstructive pulmonary disease and emphysema, use of inhaled drugs in the last 12 months, allergic disorders, and smoking habits. Participants were also asked whether they had any nasal allergies including hayfever.

Current asthma was defined as self-reported asthma with at least one respiratory symptom (wheezing, nocturnal tightness in the chest, attacks of shortness of breath following strenuous activity, at rest or at night-time) in the last 12 months and/or use of asthma medication. Chronic obstructive pulmonary disease (COPD) and emphysema were defined by self-reported physician diagnosis, whereas hayfever was defined by self-report of hayfever or other allergies with similar symptoms in the last 12 months.

2.3 | Anthropometry

Participant height and weight were measured by trained health technicians and used to calculate body mass index (BMI) (weight [kg]/height [m²]). BMI was classified in accordance with World Health Organization categories: underweight (<18.5 kg/m²), normal weight (18.5-25 kg/m²), overweight (>25-30 kg/m²), obese (>30-35 kg/m²), and very obese (>35 kg/m²).

2.4 | Smoking

A smoker was defined as someone who had smoked at least 20 packs of cigarettes or 360 g of tobacco throughout life, or at least one cigarette a day or one cigar a week for at least 1 year.
on smoking habits during the month previous to the study, smokers were further divided into current and ex-smokers. Never-smokers were defined as subjects who never smoked or smoked less than the amount used above to define smokers. Additional questions were asked about age of smoking debut, whether they had stopped or cut down, and the amount currently/previousy smoked. The mean number of cigarettes, cigars, cigarillos, and grams of pipe tobacco smoked per day was used to quantify exposure in current smokers. Lifetime exposure to smoking was calculated in pack-years (1 pack-year equals smoking 20 cigarettes (one pack) per day for 1 year). Time since stopped smoking was defined as the period of time (in years) since ex-smokers had quit smoking.

### 2.5 Immunoglobulin E (IgE) sensitization and total IgE

IgE analysis was performed in a single central laboratory (AMC Amsterdam) by using the ImmunoCAP system (Thermo Fisher Scientific). In all centres, total IgE and specific IgE were measured against Dermatophagoides pteronyssinus (house dust mite), timothy grass, and cat. IgE sensitization was defined as the presence of IgE titres for a specific allergen ≥0.35 kU/L. Groupwise differences were studied regarding F<sub>E</sub>NO in different combinations of specific IgE allergens. Group 1: non-sensitized (mite-, cat-, grass-negative); group 2: only sensitized to grass; group 3: only sensitized to perennial allergens (mite- and/or cat-positive); and group 4: sensitized to both grass and perennial allergens (mite- and/or cat-positive).

### 2.6 Measurements of exhaled nitric oxide

Nitric oxide measurements were performed in accordance with the recommendations of the American Thoracic Society, with the exception that they were performed as single measurements. Patients were instructed to avoid smoking, eating or drinking, and strenuous exercise in the hour before the measurement. F<sub>E</sub>NO values were measured with an electrochemical analyser (NIOX MINO; Aerocrine AB) at an expiratory flow rate of 50 mL/s. This device detects exhaled NO values from 5 to 300 ppb. Values below 5 ppb (the lower limit of detection of the device) were recorded in 12 subjects, and these received an arbitrary value of 3.5 ppb (5 divided by √2). No values above 300 ppb were recorded in our material.

### 2.7 Statistical methods

All analyses were performed using Stata 14.2 (StataCorp). The results are described as means, geometric mean values, or back-transformed β-coefficient with 95% confidence intervals (CI). Logarithmic transformation was performed for variables with right-skewed distribution (F<sub>E</sub>NO, total IgE, current cigarettes per day, cigarette packs/10 y, and ex-pack/y).

We have used known determinants of F<sub>E</sub>NO from the literature as predictors of F<sub>E</sub>NO in our models: age, gender, height, BMI, smoking, asthma, and allergy.

### Table 1: Baseline characteristics of participants divided by whole sample, men and women

<table>
<thead>
<tr>
<th>Variable</th>
<th>Men (n = 1969)</th>
<th>Women (n = 1912)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F&lt;sub&gt;E&lt;/sub&gt;NO (ppb)* (geometric), 95% CI</td>
<td>18.2 (17.7, 18.6)</td>
<td>15.0 (14.7, 15.4)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>54.4 (±7.1)</td>
<td>53.9 (±7.0)</td>
</tr>
<tr>
<td>Age (quartile), n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1: 39.7–48.3 y</td>
<td>475 (24.1)</td>
<td>504 (26.4)</td>
</tr>
<tr>
<td>Q2: 48.4–54.5 y</td>
<td>477 (24.2)</td>
<td>510 (26.7)</td>
</tr>
<tr>
<td>Q3: 54.6–60.2 y</td>
<td>505 (25.7)</td>
<td>448 (23.4)</td>
</tr>
<tr>
<td>Q4: 60.3–67.6 y</td>
<td>512 (26.0)</td>
<td>450 (23.5)</td>
</tr>
<tr>
<td>Height (m), (±SD)</td>
<td>1.77 (±0.07)</td>
<td>1.64 (±0.07)</td>
</tr>
<tr>
<td>Weight (kg), (±SD)</td>
<td>85.7 (±14.6)</td>
<td>70.9 (±14.4)</td>
</tr>
<tr>
<td>BMI (kg/m&lt;sup&gt;2&lt;/sup&gt;), n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;18.5</td>
<td>7 (0.4)</td>
<td>21 (1.1)</td>
</tr>
<tr>
<td>&gt;18.5–25</td>
<td>585 (29.8)</td>
<td>857 (44.9)</td>
</tr>
<tr>
<td>&gt;25–30</td>
<td>956 (48.6)</td>
<td>630 (33.0)</td>
</tr>
<tr>
<td>&gt;30–35</td>
<td>332 (16.9)</td>
<td>272 (14.3)</td>
</tr>
<tr>
<td>≥35</td>
<td>86 (4.4)</td>
<td>127 (6.7)</td>
</tr>
<tr>
<td>Total IgE (kU/L) (geometric), 95% CI</td>
<td>28.3 (26.4, 30.4)</td>
<td>20.6 (19.1, 22.2)</td>
</tr>
<tr>
<td>IgE sensitized to different allergen, n (%):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-sensitized</td>
<td>1,467 (77.5)</td>
<td>1,482 (81.0)</td>
</tr>
<tr>
<td>Sensitized only to grass pollen</td>
<td>132 (7.0)</td>
<td>130 (7.1)</td>
</tr>
<tr>
<td>Sensitized only to perennial allergens</td>
<td>188 (9.9)</td>
<td>127 (7.0)</td>
</tr>
<tr>
<td>Sensitized both to grass pollen and perennial allergens</td>
<td>107 (5.6)</td>
<td>90 (4.9)</td>
</tr>
<tr>
<td>Hayfever, yes, n (%)</td>
<td>478 (24.3)</td>
<td>536 (28.1)</td>
</tr>
<tr>
<td>Smoking: n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never-smoker</td>
<td>782 (39.8)</td>
<td>898 (47.1)</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>798 (40.6)</td>
<td>681 (35.8)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>386 (19.6)</td>
<td>326 (17.1)</td>
</tr>
<tr>
<td>Current smokers: (geometric mean, 95% CI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number cig/d</td>
<td>11.0 (10.0, 12.1)</td>
<td>8.2 (7.4, 9.1)</td>
</tr>
<tr>
<td>Pack-years</td>
<td>25.7 (23.6, 27.9)</td>
<td>18.0 (16.4, 19.4)</td>
</tr>
<tr>
<td>Ex-smokers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time since stopped (±SD)</td>
<td>17.7 (±11.6)</td>
<td>18.4 (±11.1)</td>
</tr>
<tr>
<td>Smoking (y) Pack-years (95% CI)</td>
<td>8.8 (8.0, 9.8)</td>
<td>6.9 (6.3, 7.6)</td>
</tr>
</tbody>
</table>

Note. Abbreviations: BMI: body mass index, F<sub>E</sub>NO: fractional exhaled nitric oxide, IgE: immunoglobulin E.

Values: n [%] and mean (±SD).
Perennial allergens (mite- and/or cat-positive).

All analyses were performed for men and women separately. Bivariate linear regression analyses were used to assess the cross-sectional associations between F<sub>E</sub>NO level. Age was divided into age quartiles (39.7–48.3, 48.4–54.5, 54.6–60.2 and 60.3–67.6 years),
height, weight, BMI group, smoking history, total IgE level, IgE sensitization to mite, cat, and grass group, and hay fever. Further, bivariate linear regression analysis was performed in relation to the number of cigarettes smoked daily and pack-years, and in ex-smokers, we analysed $F_{\text{ENO}}$ levels in relation to smoked pack-years and time since stopped smoking.

Multiple linear regression analyses were adjusted for age quartile, height, BMI group, smoking habits (three strata: never-, ex-, current smokers), IgE sensitization profile, hay fever, study centre, and self-reported asthma. Interaction analyses between sex and age group, IgE allergen group (mite, cat, and grass), smoking group, current cigarettes, time since stopped smoking, and ex-pack-years were performed on $F_{\text{ENO}}$ as outcome.

These multiple linear regression analyses were also tested for consistency when using a mixed linear model where grouping was done according to study centre.19

The regression coefficient for the predictor variable of interest ($\log F_{\text{ENO}}$) was back-transformed when the independent variable was normally distributed, by taking the antilog of the estimated transformed $F_{\text{ENO}}$ value. Coefficients should be interpreted as the % change of $F_{\text{ENO}}$ when the independent variables change one unit or in relation to the reference group (for example smokers vs never-smokers). When both the dependent and independent variables were log-transformed, no reverse transformation was performed (1% increase in the independent variable gave the coefficient per cent increase in the dependent variables).

A $P$ value of $<0.05$ was considered statistically significant.

### 2.8 | Ethics

Informed consent was obtained from all participants prior to inclusion in ECRHS III. Each study centre obtained approval for the study from their regional committee of medical research ethics in accordance with national legislation.

### 3 | RESULTS

In total, 1912 (49.3%) of the 3881 participants were women. The mean age was 54.4 years for men and 53.9 years for women. Exhaled NO levels were higher in men than in women: (geometric mean) 18.2 (95% confidence interval (CI): 17.7-18.6) vs 15.0 (14.7-15.4) ppb, $P$ < 0.001. Baseline characteristics by sex are given in Table 1.

### 3.1 | Exhaled NO in relation to anthropometric characteristics

Men had a 21.3% higher level of $F_{\text{ENO}}$ compared with women. Age was positively associated with $F_{\text{ENO}}$ level. Men in the highest age quartile (60.3-67.6 years) had 19.2 ppb (95% CI: 18.3, 20.2) higher $F_{\text{ENO}}$ than subjects in the lowest age quartile (39.7-48.3 years) $P$ = 0.02. Women in the two highest age quartiles (54.6-60.2 and 60.3-67.6 years) had 15.4 ppb (14.7, 16.2), $P$ = 0.03 and 16.4 ppb ($P$ = 0.03) and current smokers ($12.6$ ppb [11.9, 13.3], $P$ < 0.001). Women who never smoked had significantly higher levels of $F_{\text{ENO}}$ (geometric mean [95% CI]) (16.3 ppb [15.8, 16.8]) than current smokers (10.9 ppb [10.4, 11.5], $P$ < 0.001) while ex-smokers (12.6 ppb [11.9, 13.3], $P$ < 0.001).

![FIGURE 1](image1.png)  
**FIGURE 1** $F_{\text{ENO}}$ levels (geometric mean, 95% CI) in age groups, presented separately for males and females. Abbreviations: CI, confidence interval; $F_{\text{ENO}}$, fractional exhaled nitric oxide; GM, geometric mean

![FIGURE 2](image2.png)  
**FIGURE 2** $F_{\text{ENO}}$ levels (geometric mean, 95% CI) in never-, ex- and current smokers, presented separately for males and females. Abbreviations: CI, confidence interval; $F_{\text{ENO}}$, fractional exhaled nitric oxide; GM, geometric mean
no difference between never- and ex-smokers could be found ($P = 0.19$, Figure 2 and Table S1). No significant interaction with gender was found on the relation between ex-smoking and $F_{e}NO$ ($P = 0.44$).

For men that were ex-smokers, there was a positive association between $F_{e}NO$ and the time since they had stopped smoking (coefficient by 10 years: $1.05$ [95% CI: 1.02, 1.08]), no significant association was seen in women. Further, no association was found between smoking history (pack-year) and $F_{e}NO$ level in ex-smoking men and women. Among current smokers, a significant negative association was found between $F_{e}NO$ level and number of cigarettes per day and pack-years (both $P = 0.001$) in both men and women (Table 2).

### 3.3 Exhaled NO and the relation to IgE sensitization, mite, cat, and grass exposure

Total IgE was associated with higher levels of $F_{e}NO$ in women ($P = 0.02$), but not in men ($P = 0.91$). Men and women sensitized to mite, cat, or grass had higher levels of $F_{e}NO$ compared with non-sensitized subjects ($P < 0.001$, separate analyses for one allergen at a time).

When studying the allergens in different combinations, men sensitized to grass had 13% (95% CI; 3, 25%) higher $F_{e}NO$ levels than non-sensitized subjects; for perennial allergens, (cat and mite) the levels were 11% (95% CI; 2, 20%) higher, and for grass and perennial allergens 33% (95% CI; 19, 48%) higher. Women sensitized to grass had 11% (95% CI; 2, 22%) higher $F_{e}NO$ levels than those non-sensitized; for perennial allergens (cat and mite), the levels were 15% (95% CI; 5, 26%) higher, and for grass and perennial allergens 35% (95% CI; 22, 51%) higher (Figure 3 and Table S1).

### 3.4 Multivariate model of determinants of exhaled NO

When we stratified by sex and age groups, the oldest men (60.3-67.6 years) had 9% (95% CI; 1%-16%, $P = 0.02$) higher $F_{e}NO$ values than men in the lowest age quartile (39.7-48.3 years). Women in the two highest age quartiles (54.5-60.2 and 60.3-67.7 years) had

![Figure 3](https://example.com/figure3.png)

**FIGURE 3** $F_{e}NO$ levels (geometric mean, 95% CI) in relation to sex in non-sensitized, sensitized to grass, sensitized to perennial allergens (mite- and/or cat-positive), sensitized to both grass and perennial allergens (mite- and/or cat-positive). Abbreviations: CI, confidence interval; $F_{e}NO$, fractional exhaled nitric oxide; GM, geometric mean.
Men had 8% (95% CI; 1%-14%; \( P = 0.02 \)) and 13% (95% CI; 6%-21%; \( P < 0.001 \)) higher F\(_\text{E}\)NO values, respectively, than those in the lowest age quartile (39.7-48.3 years). Height was related to 8% (95% CI; 5%-12%; \( P < 0.001 \)) higher F\(_\text{E}\)NO level in men and 5% (95% CI; 1%-9%; \( P = 0.008 \)) higher F\(_\text{E}\)NO levels in women (Figure 4 and Table S2). BMI was not significantly related to F\(_\text{E}\)NO levels (data not shown).

Men who smoked had 37% (95% CI; 32%-41%) lower F\(_\text{E}\)NO levels and women 30% (95% CI; 25%-34%) lower compared with never-smokers (\( P < 0.001 \) for both). Among ex-smokers, men had 5% lower F\(_\text{E}\)NO levels compared with never-smokers (\( P = 0.02 \)). No significant associations were seen between F\(_\text{E}\)NO and women who were ex-smokers compared with never-smokers.

Women who were sensitized to both grass and perennial allergens had higher F\(_\text{E}\)NO levels compared with non-sensitized subjects (26% 95% CI; 14%-39%) and 29% (95% CI; 16%-43%), \( P < 0.001 \) for both). Only men showed significant effects of grass sensitization on F\(_\text{E}\)NO (\( P = 0.02 \) in men, \( P = 0.13 \) in women). Women who were sensitized to perennial allergens (mite and/or cat) had higher levels of F\(_\text{E}\)NO than non-sensitized women (\( P = 0.003 \)). No significant association was seen among men (\( P = 0.09 \)) (Figure 4 and Table S2). No significant association was seen between hayfever and F\(_\text{E}\)NO levels (data not shown). These results were consistent, both regarding significance and size of the effects, in a mixed linear model with subjects grouped by centre (data not shown).

The only significant interaction between sex and other predictors (age group, IgE allergen group (mite, cat, and grass), smoking group, current cigarettes, time since stopped smoking, and ex-pack-years) was between sex and current smoking (\( P < 0.05 \)), both in a multiple linear regression model or mixed model (Table S3).

4 | DISCUSSION

We have found that F\(_\text{E}\)NO levels were about 21% higher in males than females in this large European multi-centre study of healthy, middle-aged subjects. Similar determinants of F\(_\text{E}\)NO were found to be associated with higher F\(_\text{E}\)NO levels in both males and females with increased height and IgE sensitization. Current smoking was found to be associated with lower F\(_\text{E}\)NO levels and the size of this effect was larger in men than women. Higher age related to higher F\(_\text{E}\)NO levels, and this effect was seen at a lower age in women than men. Previous smoking was related to a small, but significant decrease in F\(_\text{E}\)NO levels in men.

In our study, F\(_\text{E}\)NO levels were 21% higher in men than women (18.2 vs 15.0 ppb). Several previous studies have also reported
an association between increased $F_2$NO levels and male sex.\textsuperscript{20-23} Kim et al\textsuperscript{22} reported, based on data from 166 healthy Korean adults (aged 20-68 years), that men had 27% higher $F_2$NO levels than women (35.7 vs 26.0 ppb). Taylor et al\textsuperscript{23} studied 895 healthy adults at age 32 years and found that men had approximately 25% higher $F_2$NO levels than women (15.5 vs 11.6 ppb). A similar size of the sex difference has also been reported in asthmatic subjects\textsuperscript{21,24} for example, Al-shamkhi et al\textsuperscript{21} reported that in 557 subjects with asthma from the Swedish GA2LEN study, men had 32% higher $F_2$NO levels than women (24.0 vs 16.4 ppb). However, not all studies have been able to detect sex differences in $F_2$NO.\textsuperscript{25,26} Olin et al\textsuperscript{25} studied 2200 randomly selected healthy adults, aged 25-75 years, and reported that sex was not independently associated with $F_2$NO. In a recent study by Högman et al\textsuperscript{26} on 433 healthy subjects, age 7-78 years, a significant sex effect on $F_2$NO levels could be reported only for the middle age group, 20-49 years. The mechanism of how sex affects $F_2$NO is not fully understood, but a few hypotheses are worth mentioning. Greater height\textsuperscript{27} could explain larger lung and airway size, leading to larger surface of the airway mucosa, larger airway calibre, and increased NO release.\textsuperscript{20,28} However, this could only partly explain our differences, as male sex was still associated with increased $F_2$NO level after adjustment for height. Other potential mechanisms might be genetic differences\textsuperscript{29} and effects of oestrogen.\textsuperscript{30,31}

Our study showed a significantly increased level of $F_2$NO after age 55 in women and age 60 in men. Jacinto et al\textsuperscript{11} reported an increase in $F_2$NO level in the age group 14-16 years, depending on sex, based on data from the National Health and Nutrition Examination Survey (NHANES). Beyond this age, $F_2$NO plateaus and shows stable values until age 45 years in women and age 59 years in men, when it starts to increase. There were some differences between our study and the study of Jacinto et al regarding studied population, as Jacinto et al excluded subjects with hayfever, previous/current smoking, and suspicion of inflammatory diseases. These changes in $F_2$NO seem related to somatic growth in childhood, which ends in the upper teens. The increase from middle age and up may be primarily related to structural changes in the lungs, for example loss of alveolar elastic recoil and alveolar surface area\textsuperscript{22} and reduced alveolar-capillary diffusion of NO.\textsuperscript{26,32} This process probably starts earlier in women than men and to some extent explains the present findings and the findings of Jacinto et al. However, a recent study on 303 healthy, non-smoking seniors, aged over 65 years (with a mean age of 85 years), found no difference in $F_2$NO values between males and females,\textsuperscript{34} so a cohort study covering all ages would be of interest to fully understand the relation of $F_2$NO to age and sex.

In the present study, current and ex-smokers showed lower $F_2$NO levels compared with never-smokers. This result is in accordance with a study of Xu et al\textsuperscript{35} on 11 160 subjects from NHANES; they showed that active smoking, measured by self-report, among healthy and asthmatic subjects, was associated with 37% and 45% lower $F_2$NO levels, respectively. In the present study, there was a significant interaction between sex and current smoking. However, this is likely to be due to the fact that men smoked more, as no interaction with sex on $F_2$NO levels was found in relation to number of smoked cigarettes. This argues against the idea that women might be more sensitive to smoking in terms of $F_2$NO reduction.

Further, there was a relation between previous smoking and $F_2$NO in men in form of a 5% reduction in $F_2$NO levels. Other authors diverge on this matter, reporting a decrease,\textsuperscript{36} no effect,\textsuperscript{25,37} or an increase of $F_2$NO levels.\textsuperscript{38} We also found that in men, there was an association with time since they stopped smoking, suggesting that the decrease might be seen among all men who stopped smoking recently. However, this effect was not found in women. Several mechanisms on the link of current smoking with decreased $F_2$NO have been proposed: down-regulation of enzymatic NO formation in the bronchial compartment, as well as in the oropharyngeal compartment.\textsuperscript{39} Interferon gamma, which is present in normal airways, seems to be down-regulated in smokers, which leads to a decreased expression of iNOS in the human respiratory epithelium;\textsuperscript{39} another potential mechanism is that smoke contains high levels of NO, which has been found to have an inhibitory feedback effect on NOS.\textsuperscript{40}

Allergic sensitization was associated with higher levels of $F_2$NO, especially when subjects were sensitized to both grass and mite and/or cat allergens. IgE sensitization has in other studies been shown to be related to higher $F_2$NO levels,\textsuperscript{14,38} and a degree of IgE sensitization has been reported to relate to $F_2$NO levels, either when assessed as titres of IgE\textsuperscript{14} or number of sensitizations/types of allergens. In a study by Yau et al on 1321 healthy children, significant positive associations were found between $F_2$NO and specific allergens, and between $F_2$NO and the number of sensitizations. However, Silvestri et al\textsuperscript{41} studied 112 children with stable, mild intermittent asthma and no differences were seen between $F_2$NO levels and mono- and poly-sensitized subjects.

We found no significant association between BMI and $F_2$NO levels in the multivariate model. These results indicate that BMI has no effect on $F_2$NO levels and that $F_2$NO is affected to a greater extent by confounders such as sex, age, and height. Our results are in accordance with a study by Kim et al,\textsuperscript{42} a cross-sectional study on 117 healthy subjects, aged 20-68 years, which could not find any significant association between BMI and $F_2$NO. Similar results were also seen in a study on healthy children.\textsuperscript{43} However, some studies have shown contradictory results. Studies by De Winter-de Groot et al,\textsuperscript{44} on 24 healthy non-smoking subjects, and by Kazaks et al,\textsuperscript{45} on 25 healthy subjects, reported a significant positive association between BMI and $F_2$NO.

The main strength of the current report is the use of a large, multi-centre, general population sample with high-quality and standardized measurements of exhaled NO, using the same type of device in all centres. Nevertheless, some limitations must be taken into consideration. ECRHS III does not include measures of bronchial responsiveness. We excluded subjects with self-reported asthma and/or asthma symptoms in the 12 months preceding the questionnaire. Thus, it is possible that subjects with no or minimal symptoms in the most recent 12 months might be enrolled as healthy subjects.
However, subjects receiving medication were excluded, which would argue against subjects with asthma having been enrolled as healthy subjects. Also, having subjects from different centres and geographical areas is a strength indicating that these findings could be valid in the general population. The present population is recruited from the random sample of ECRHS. However due to the long follow-up time and that this is the second follow-up, selection bias cannot be ruled out.

Our data confirm a difference in $F_{E}NO$ levels between men and women. Present algorithm for clinical interpretation $F_{E}NO$ does not take sex into account. $F_{E}NO$ started increasing at lower age in women than in men, suggesting that interpretation of $F_{E}NO$ levels in adults aged over 50 years should take into account both age and sex. Similar determinants and effect sizes of different confounders in adults aged over 50 years should take into account both age and sex.

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AUTHOR CONTRIBUTIONS

E. Nerpin, PhD, C. Janson, PhD, and A. Malinovski, PhD conceived, designed, or analysed and interpreted the data; drafted the article or revised it; provided intellectual content of critical importance to the work described; and are accountable for ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. M. Olivieri, PhD, T. Gislason, PhD, A. C. Olin, PhD, R. Nielsen, PhD, A. Johannessen, PhD, D. S. Ferreira, PhD, R. Jögi, B. Forsberg, J.P. Zock, T. Sigsgaard, D. Jarvis, PhD, A. Marcon, PhD, I. Pin, PhD, A. Corsico, P. Demoly, D. Nowak, J. Eeyler, J. Heinric, PhD, R. Bono, PhD, and B. Leynaert, PhD conceived, designed, or analysed and interpreted the data; drafted the article or revised it; and provided intellectual content of critical importance to the work described. L. Cazzoletti and S. Accordini conceived, designed, or analysed and interpreted the data; are accountable for ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.
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