

# Passive and active smoking and exhaled nitric oxide levels according to asthma and atopy in adults

Rachel Nadif, PhD\*†; Regis Matran, MD‡§; Jean Maccario, PU\*¶; Magali Bechet, Engineer\*†; Nicole Le Moual, PhD\*†; Pierre Scheinmann, MD||; Jean Bousquet, MD\*†; Francine Kauffmann, MD\*†; and Isabelle Pin, MD#; on behalf of the EGEA (Epidemiological study on the Genetics and Environment of Asthma, bronchial hyperresponsiveness and atopy)

**Background:** No data are available regarding the utility of fractional exhaled nitric oxide (FeNO) level in guiding therapy in smoking asthmatic patients. Identification of the effect of smoking in a large sample is needed.

**Objective:** To study the association between smoking and FeNO level according to current asthma and atopy status in adults from the French EGEA (Epidemiological study on the Genetics and Environment of Asthma, bronchial hyperresponsiveness and atopy).

**Methods:** Levels of FeNO were measured at 50 mL/s in 654 adults (268 asthmatic participants). Active smoking and environmental tobacco smoke (ETS) exposure at home, at work, and during leisure activities were recorded. Participants were categorized as having no exposure to ETS, mild exposure (ETS <2 h/d), and noticeable exposure (ETS ≥2 h/d). Multivariate analyses were performed, with adjustment for age, sex, height, and center.

**Results:** Mean adjusted FeNO values increased with asthma (15.1 vs 19.5 ppb), atopy (14.2 vs 18.9 ppb), and eosinophilia (15.8 vs 24.8 ppb) ( $P < .001$  for all). Mean FeNO levels decreased with smoking (18.4, 17.5, and 14.5 ppb in nonsmokers, ex-smokers, and current smokers, respectively;  $P$  for trend = .001). The association with smoking was observed in nonasthmatic and asthmatic participants, especially in atopic asthmatic participants. Multivariate analyses showed that ETS exposure of at least 2 h/d and active smoking were negatively and significantly associated with FeNO levels independent of age, sex, height, and center in nonasthmatic participants (mean [SE],  $-0.13$  [0.06],  $P = .03$  and  $-0.10$  [0.03],  $P < .001$ ) and in asthmatic participants (mean [SE],  $-0.18$  [0.07],  $P = .01$  and  $-0.14$  [0.04],  $P = .02$ ).

**Conclusions:** Active and passive smoking decreased FeNO levels in adults. Careful consideration of asthma, atopy, and active and passive smoking are needed to interpret FeNO values.

*Ann Allergy Asthma Immunol.* 2010;104:385–393.

## INTRODUCTION

The noninvasive measure of fractional concentration of endogenous exhaled nitric oxide (FeNO) has great potential in the clinical setting and in epidemiologic study as a biomarker of pulmonary inflammation. However, methodological aspects and insufficient knowledge regarding its determinants currently limit its applicability as a good “inflammometer” to guide the management of asthma.<sup>1,2</sup> In a recent review,<sup>3</sup>

differences in design and methodological issues and the lack of consideration of environmental and host factors have been proposed to explain that FeNO-based algorithms are less successful at guiding asthma therapy than are sputum eosinophils. As underlined by the author, no data are available regarding the utility of FeNO to guide therapy in smoking asthmatic patients, and identification of the effect of smoking in large samples is needed.

Epidemiologic studies led to conflicting results regarding the complex associations of FeNO with smoking, asthma, and atopy. FeNO was negatively associated with current smoking in atopic individuals only<sup>4</sup> and was positively associated with asthma symptoms in the last month in never-smokers only.<sup>5</sup> Whereas recent observations by Malinovschi et al<sup>6</sup> suggest that increased FeNO levels in asthma (allergic or not) may be restricted to never smokers, those by Michils et al<sup>7</sup> show an association of FeNO level with asthma control, even in smokers. The debate regarding the clinical relevance of monitoring FeNO levels in asthma remains open. Further studies are needed to disentangle the complex relationships of atopy, smoking, and asthma, particularly in adults.

Although the decrease in FeNO levels in relation to active smoking is established, few studies<sup>7,8</sup> have been conducted on

**Affiliations:** \* Inserm, CESP Centre for Research in Epidemiology and Population Health, Respiratory and Environmental Epidemiology Team U1018, Villejuif, France; † Université Paris Sud 11, UMRS 1018, Villejuif, France; ‡ Univ Lille Nord de France, Lille, France; § CHU, Lille, France; ¶ Université Paris-Descartes, Paris, France; || Hôpital Necker-Enfants Malades, Pneumo-Allergologie, Paris, France; # CHU de Grenoble; Inserm, U823; Université Joseph Fourier, Grenoble, France.

**Disclosures:** Authors have nothing to disclose.

**Funding Sources:** This research was funded in part by PHRC-Paris, ANR 05-SEST-020-02/05-9-97, ANR-06-CEBS, Merck Sharp & Dohme, and the GA2LEN project (Global Allergy and Asthma European Network).

Received for publication February 8, 2010; Received in revised form March 13, 2010; Accepted for publication March 18, 2010.

© 2010 American College of Allergy, Asthma & Immunology.

Published by Elsevier Inc. All rights reserved.

doi:10.1016/j.anai.2010.03.013

the effect of smoking in asthmatic patients. Studies<sup>9,10</sup> in infants and children suggest that in utero exposure to tobacco and environmental tobacco smoke (ETS) decrease FeNO levels, but it is unknown whether usual exposure to ETS modifies FeNO levels in adults. Understanding the effect of ETS in asthmatic individuals across the lifespan is of particular relevance.

The overall hypothesis of this study is that the association between asthma and FeNO level varies according to active and passive smoking. Using data from the EGEA (Epidemiological study on the Genetics and Environment of Asthma, bronchial hyperresponsiveness and atopy), the aim of the present study was to investigate the effects of active and passive smoking on FeNO levels in adults according to current asthma and atopy status, simultaneously taking into account host factors (age, sex, and height).

## METHODS

### Study Design

The EGEA combines a case-control and family study of asthma (<http://ifr69.vjf.inserm.fr/~egeanet/>). Participants were recruited between 1991 and 1995, and the protocol and descriptive

characteristics have been described elsewhere.<sup>11,12</sup> The present cross-sectional analysis is based on FeNO measurements recorded during follow-up conducted between 2003 and 2007.<sup>13</sup> The studied population included 654 adults (325 men and 329 women; 299 in Paris, 63 in Montpellier [France], and 292 in Grenoble) with data on current asthma, atopy, and smoking (Fig 1). Ethical approval was obtained from the relevant committees (Cochin Port-Royal Hospital and Necker-Enfants Malades Hospital, Paris), and written informed consent was provided by all the participants. A quality management approach was followed for implementation of EGEA2 data collection, including strong efforts to standardize the examination procedures, which led to ISO 9001:2000 certification (<http://www.afaq.org/certification=262711141114>).

### Phenotypes

“Ever asthma” was defined by a positive answer to 1 of the 2 following questions: “Have you ever had attacks of breathlessness at rest with wheezing?” “Have you ever had asthma attacks?” Of individuals with asthma, “current asthma” included those who reported respiratory symptoms in the past

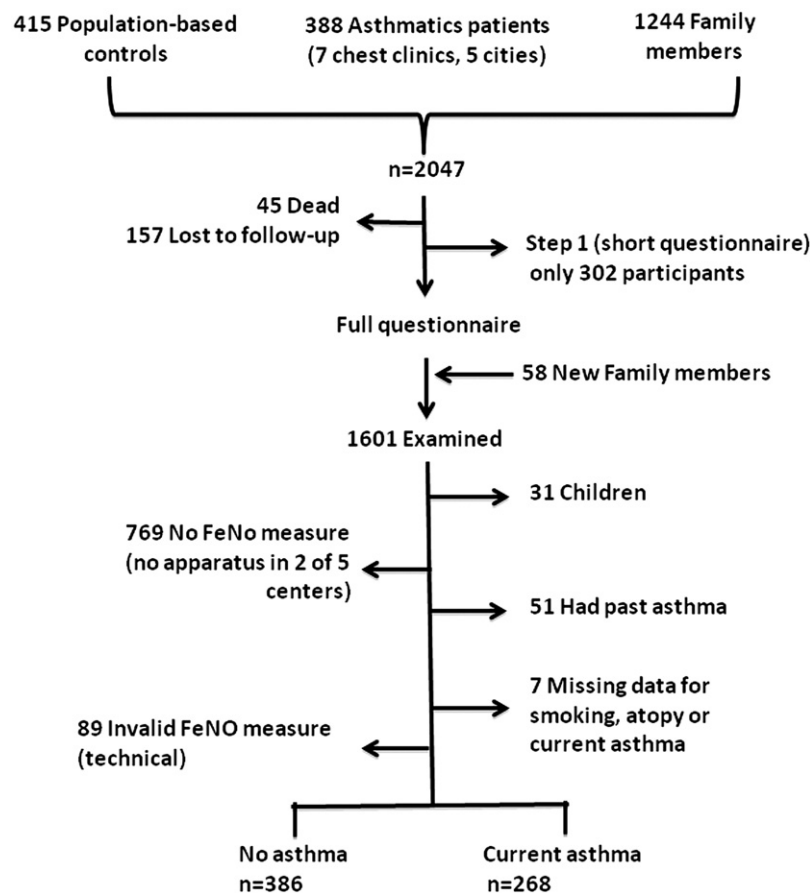


Figure 1. Flowchart of the studied population. FeNO indicates fractional exhaled nitric oxide.

12 months (wheeze; nocturnal chest tightness; attacks of breathlessness after activity, at rest, or at night; or asthma attacks) or the use of inhaled and/or oral medicines because of breathing problems. Asthmatic participants without current asthma were excluded from the analyses. Asthma severity was defined based on Global Initiative for Asthma 2006 guidelines combining asthma symptoms and treatment, as previously used.<sup>14</sup> Atopy was defined by a mean wheal diameter at least 3 mm greater than the negative control in at least 1 of 11 aeroallergens (cat, *Dermatophagoides pteronyssinus*, *Blattella germanica*, olive, birch, *Parietaria judaica*, timothy grass, ragweed pollen, *Aspergillus*, *Cladosporium herbarum*, and *Alternaria tenuis*). Symptomatic allergic rhinitis was defined by a positive answer to the following question: “Have you had sneezing problems or a runny nose when you were in contact with animals, dust, hay, or cut flowers in the past 12 months?” Eosinophilia was defined from white blood cell counts as an eosinophil count greater than 5%. Lung function tests with methacholine challenge were performed according to standardized procedures. “Smokers” were defined as individuals who reported currently smoking cigarettes, pipes, cigarillos, or cigars at the time of the study and for at least 1 year. “Ex-smokers” were individuals who smoked daily and stopped smoking for at least 4 weeks. “Nonsmokers” were those who had never smoked. The daily consumption of tobacco was evaluated considering 1 cigarette, 1 cigarillo, and 1 cigar equal to 1, 2, and 5 g, respectively. ETS combined exposure at home (currently living with  $\geq 1$  smokers), at work (working in a smoky area  $\geq 1$  h/d), and during leisure activities ( $\geq 1$  h/d). Participants were categorized as having no exposure to ETS (ETS–), mild exposure (ETS <2 h/d), and noticeable exposure (ETS  $\geq 2$  h/d).

#### FeNO Measurements

Online (Paris and Montpellier) and offline (Grenoble) measurements were realized according to American Thoracic Society/European Respiratory Society recommendations for standardized procedures before other pulmonary function tests.<sup>15</sup> Participants avoided food and beverages (including alcohol) and smoking tobacco for at least 1 hour before testing. Upper and lower respiratory tract infections within 4 weeks were recorded. Participants, seated comfortably in the upright position without a nose clip, were asked to exhale at a constant flow against an oral pressure of at least 5 cm of H<sub>2</sub>O. Measurements were taken for at least 6 seconds. Three NO analyzers were used in this study: Endono 8000 (Seres, Aix-en-Provence, France) in Paris, Aerocrine (Aerocrine AB; Solna, Sweden) in Montpellier, and Topaze 2020 (Cosma SA, Igny, France) in Grenoble. The 3 analyzers were calibrated with gas containing NO (Air Liquide, Paris, France). For Endono 8000 and Topaze 2020 analyzers, the flow rate was calibrated daily. For the Aerocrine analyzer, the flow rate was calibrated every 13 days according to manufacturer standards. Scrubbed air (0 ppb of NO) was inhaled before measurements. Repeated reproducible exhalations were per-

formed to obtain at least 2 NO plateau values that agree within 10% of each other. Depending on the analyzer, 2 or 3 measurements at the 50-mL/s exhalation flow rate were recorded. In Grenoble (offline method) and Montpellier (online method), 3 measurements performed only at the 50-mL/s flow rate were recorded. In Paris (online method), measurements were performed at 3 exhalation flow rates: 25, 50, and 100 mL/s, and only 2 measurements were recorded at each flow rate to avoid tiring out the participants. Measurements of FeNO at the 50-mL/s flow rate on each of the 3 analyzers were combined. Participants with only 1 FeNO measurement or poor calibration were excluded from the analyses (5%).

#### Statistical Analysis

Because of skewness of distributions, analyses were performed on log<sub>10</sub> (FeNO). In the main analysis, all the models were run with age, sex, height, and center as covariates. Furthermore, due to familial aggregation of the data (654 individuals from 329 families), regression analyses of linear models on log<sub>10</sub> (FeNO) were performed using generalized estimating equations (MIXED procedure with asymptotic structure covariance).<sup>16</sup> All the analyses were performed using SAS version 9.1 (SAS Institute Inc, Cary, North Carolina).

## RESULTS

The overall characteristics of the 654 adults according to current asthma status are summarized in Table 1. As expected, compared with nonasthmatic participants, asthmatic participants had a significantly lower mean forced expiratory volume in 1 second (107.3% vs 96.4% of predicted,  $P < .001$ ); had more frequent bronchial hyperresponsiveness (odds ratio [OR], 6.5; 95% confidence interval [CI], 4.1–10.1), atopy (OR, 5.5; 95% CI, 3.7–8.0), and eosinophilia (OR, 3.5; 95% CI, 2.0–5.9); and more often reported allergic rhinitis (OR, 3.0; 95% CI, 2.1–4.2) and respiratory tract infections in the past 4 weeks (OR, 2.4; 95% CI, 1.3–4.2) independent of age, sex, and smoking.

Mean FeNO levels were significantly higher in men than in women (16.6 vs 13.7 ppb,  $P < .001$ ), a difference of borderline significance after adjustment for age, height, and center (17.9 vs 15.8 ppb in men vs women,  $P = .07$ ). Level of FeNO was unrelated to age, even after stratification on smoking, and no significant association was found between FeNO level and ambient NO and regular alcohol consumption (data not shown). A significant variation in FeNO level according to season was observed, with mean levels of 16.8, 13.7, 15.8, and 13.5 ppb in spring (March/April/May), summer, autumn, and winter, respectively ( $P = .01$ ).

#### Associations of FeNO With Asthma, Atopy, Eosinophilia, and Allergic Rhinitis

As shown in Figure 2, FeNO increased with current asthma, atopy, and eosinophilia, and positive associations between FeNO and atopy and eosinophilia were found in nonasthmatic and asthmatic participants. According to atopy, mean FeNO values were 12.6 vs 15.2 ppb ( $P = .004$ ) and 13.6 vs 18.6 ppb

Table 1. Characteristics of the 654 Adults Included in the Analyses According to Current Asthma Status

|                                                                         | No asthma<br>(n = 386) | Asthma<br>(n = 268) |
|-------------------------------------------------------------------------|------------------------|---------------------|
| Age, mean (SD), y                                                       | 43.4 (16.1)            | 36.4 (17.0)         |
| Female sex, %                                                           | 52.8                   | 46.6                |
| Height, mean (SD), m                                                    | 1.69 (0.09)            | 1.70 (0.08)         |
| Smoking habits, %                                                       |                        |                     |
| Nonsmokers                                                              | 48.9                   | 53.0                |
| Ex-smokers                                                              | 28.0                   | 20.9                |
| Smokers                                                                 | 23.1                   | 26.1                |
| In nonsmokers                                                           |                        |                     |
| No ETS, %                                                               | 74.2 <sup>b</sup>      | 66.8                |
| Mild ETS (<2 h/d), %                                                    | 14.6                   | 18.3                |
| Noticeable ETS (≥2 h/d), %                                              | 11.2                   | 14.9                |
| In smokers                                                              |                        |                     |
| Daily tobacco consumption, mean (SD), g                                 | 10.1 (7.2)             | 8.4 (7.6)           |
| Heavy smokers, >20 g/d, %                                               | 17.1                   | 11.4                |
| In ex-smokers and current smokers                                       |                        |                     |
| Pack-years, mean (SD)                                                   | 11.8 (14.5)            | 7.5 (11.1)          |
| Asthma severity according to GINA 2006 guidelines, %                    |                        |                     |
| Intermittent                                                            | NA                     | 62.6 <sup>c</sup>   |
| Mild persistent                                                         | NA                     | 4.3                 |
| Moderate persistent                                                     | NA                     | 11.7                |
| Severe persistent                                                       | NA                     | 21.4                |
| FEV <sub>1</sub> , according to asthma severity, mean (SD), % predicted | 107.9 (16.1)           | 97.1 (17.6)         |
| Intermittent                                                            | NA                     | 102.6 (11.8)        |
| Mild persistent                                                         | NA                     | 98.6 (12.1)         |
| Moderate persistent                                                     | NA                     | 91.8 (15.1)         |
| Severe persistent                                                       | NA                     | 81.3 (22.9)         |
| FEV <sub>1</sub> <80% of predicted, %                                   | 3.1                    | 17.7                |
| Methacholine test                                                       |                        |                     |
| Participants, No. <sup>a</sup>                                          | 286                    | 176                 |
| PD <sub>20</sub> ≤4 mg, %                                               | 27.3                   | 71.0                |
| Atopy, %                                                                | 40.4                   | 80.6                |
| Eosinophilia (blood eosinophils >5%), %                                 | 6.2                    | 19.0                |
| Allergic rhinitis, %                                                    | 47.9                   | 74.2                |
| Inhaled corticosteroids, past 3 mo, %                                   | 0                      | 16.4                |
| Respiratory tract infection (past 4 wk), %                              | 5.7                    | 12.3                |

Abbreviations: ETS, environmental tobacco smoke; FEV<sub>1</sub>, forced expiratory volume in 1 second; GINA, Global Initiative for Asthma; NA, not applicable; PD<sub>20</sub>, provocation dose that caused a decrease in FEV<sub>1</sub> of 20%.

<sup>a</sup> Methacholine testing was not performed if FEV<sub>1</sub> was less than 80% of predicted.

<sup>b</sup> Not available in 2 participants due to missing values.

<sup>c</sup> Not available in 11 participants due to missing values.

( $P = .006$ ) in nonasthmatic vs asthmatic participants (Fig 2A). According to eosinophilia, FeNO values were 13.2 vs 19.2 ppb ( $P = .003$ ) and 16.2 vs 24.2 ppb ( $P < .001$ ) in nonasthmatic vs asthmatic participants (Fig 2B). Associations were similar in men and women (data not shown). Associa-

tions remained independent of age, sex, height, and center. Overall, mean adjusted FeNO values were 15.1 vs 19.5 ppb according to asthma, 14.2 vs 18.9 ppb according to atopy, 15.8 vs 24.8 ppb according to eosinophilia, and 14.6 vs 18.2 ppb according to allergic rhinitis ( $P < .001$  for all). No significant associations were found between FeNO and forced expiratory volume in 1 second or respiratory tract infections in the past 4 weeks. Stratification for asthma did not change the associations.

#### Associations of FeNO With Active Smoking

Levels of FeNO significantly decreased with active smoking independent of age, sex, height, center, and current asthma, with ex-smokers having intermediate values (Table 2). This association was similarly observed in asthmatic and nonasthmatic participants as in men and women (data not shown) and held for each center. Lower levels of FeNO were also observed with increased daily tobacco consumption or pack-years. Further stratification according to atopy showed significant associations between active smoking and FeNO levels, particularly in atopic participants, whatever the asthma status. In ex-smokers, no significant association between time since stopping smoking and FeNO level was observed, with mean values of 15.5 ppb in those who stopped smoking during the past year ( $n = 25$ ) and 17.0 ppb in those who stopped smoking for at least 1 year ( $n = 135$ ) ( $P = .4$ ).

#### Associations of FeNO Level With Passive Smoking

In nonsmokers, FeNO level was significantly associated with passive smoking, with participants exposed to ETS at least 2 h/d having the lowest mean value (19.6, 19.5, and 13.9 ppb in ETS-, mild ETS, and noticeable ETS, respectively,  $P$  for trend = .01) independent of age, sex, height, center, and current asthma. After stratification for asthma, the negative association between ETS exposure and FeNO level remained significant and was gradual in asthmatic participants (mean, 24.2, 21.5, and 15.7 ppb;  $P = .01$ ) but not in nonasthmatic participants (mean, 15.5, 18.0, and 12.6 ppb;  $P = .5$ ). The sample size did not allow further stratification according to atopy. Small numbers limited the study of ETS in ex-smokers. In ex-smokers who stopped smoking for at least 1 year, mean FeNO values were 16.5, 20.9, and 17.1 ppb in ETS- ( $n = 118$ ), mild ETS ( $n = 6$ ), and noticeable ETS ( $n = 9$ ), respectively ( $P = .5$ ).

#### Association of Active and Passive Smoking With FeNO Level According to Asthma Status

As shown in Figure 3, with all smoking categories, an overall decrease in FeNO levels was observed in relation to active and passive smoking regardless of asthma status. Results of multivariate analyses showed that noticeable ETS and active smoking were negatively and significantly associated with FeNO level independent of age, sex, height, and center, regardless of asthma status (Table 3). Taking into account ex-smokers, atopy, allergic rhinitis, eosinophilia, season, and respiratory tract infections did not change the associations. Analyses performed excluding the 5 individuals who smoked

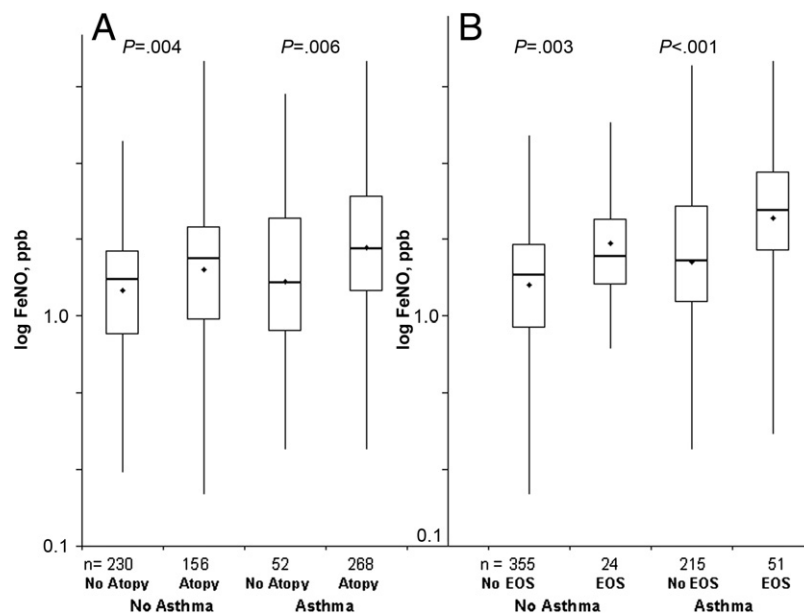


Figure 2. Associations between fractional exhaled nitric oxide (FeNO) level and atopy (A) and eosinophilia (EOS) (B) according to current asthma status. The horizontal line and the star in the middle of each box indicate the median and the mean, respectively. The top and bottom borders of each box mark the 75th and 25th percentiles, respectively. The whiskers above and below each box mark the maximum and minimum values.

in the hour before FeNO measurement and the 2 without an answer did not change any of the associations.

## DISCUSSION

The present epidemiologic study shows for the first time, to our knowledge, that not only active but also passive smoking affect FeNO levels in adults. Level of FeNO decreased with active smoking, with ex-smokers having intermediate values. The association was observed in nonasthmatic and asthmatic participants, and especially in atopic asthmatic participants. Furthermore, lower FeNO levels were found in participants exposed to ETS at least 2 h/d, an association observed in nonasthmatic and asthmatic participants. The results show that careful consideration of asthma, atopy, and active and passive smoking are needed to interpret FeNO values.

This article brings new insights into the study of the effects of active and passive smoking on FeNO level in adults taking into account asthma and atopy. In the EGEA, asthmatic participants have been well-characterized with a wide range of severity, the validity of the report of ETS exposure was good, and it was shown previously that asthma in itself did not modify the report of ETS in families of asthmatic individuals.<sup>17</sup>

Limitations of the present study include the use of 2 methods of NO measurement. Deykin et al<sup>18</sup> reported that the diagnostic power of FeNO to detect individuals with and without asthma was not affected by online or offline techniques. Careful attention was given to center heterogeneity, and analyses stratified by center were performed first. Levels of FeNO were significantly lower in Paris than in Montpellier

or Grenoble ( $P < .001$ ), with FeNO geometric means (95% CIs) of 11.3 (2.50–50.8), 21.9 (6.11–78.9), and 18.7 (8.47–41.4) ppb, respectively. The present findings are in agreement with those of Borrill et al,<sup>19</sup> who reported the same differences between analyzers when comparing FeNO measurements in individuals using 3 different analyzers in random order. Expected associations between FeNO level and asthma, atopy, allergic rhinitis, and eosinophilia were similar for each center except for Montpellier, where associations between FeNO level and asthma and eosinophilia were not significant: 20.9 vs 24.0 ppb ( $P = .4$ ) in participants without vs with asthma and 21.6 vs 22.9 ppb ( $P = .8$ ) in those without vs with eosinophilia. Furthermore, negative associations between active smoking and FeNO level were observed at each center. Overall, this suggests that differences between centers are caused by true differences between analyzers, which did not affect qualitatively the associations studied. After checking the consistency of association of FeNO level with established clinical characteristics and smoking according to centers, we checked the consistency of FeNO with genetic determinants,<sup>20</sup> and preliminary results showing similar associations in each center between FeNO level and genetic factors (not affected by measurement methods) support the validity of the measurements despite the difference in apparatus. We adjusted all the analyses on the center. Other limitations were those commonly related to cross-sectional analyses of data and to the fact that immediate practical utility could not be inferred from results obtained in the framework of an epidemiologic study.

Table 2. Associations of Active Smoking, Daily Tobacco Consumption, and Pack-Years With FeNO According to Current Asthma and Atopy Status

|                                | All, No.<br>(n = 654) | FeNO,<br>ppb <sup>a</sup> | No asthma        |                           |                  |                           |                  |                           | Asthma           |                           |                  |                           |                 |                           |
|--------------------------------|-----------------------|---------------------------|------------------|---------------------------|------------------|---------------------------|------------------|---------------------------|------------------|---------------------------|------------------|---------------------------|-----------------|---------------------------|
|                                |                       |                           | All              |                           | Atopy            |                           | No atopy         |                           | All              |                           | Atopy            |                           | No atopy        |                           |
|                                |                       |                           | No.<br>(n = 386) | FeNO,<br>ppb <sup>b</sup> | No.<br>(n = 156) | FeNO,<br>ppb <sup>b</sup> | No.<br>(n = 230) | FeNO,<br>ppb <sup>b</sup> | No.<br>(n = 268) | FeNO,<br>ppb <sup>b</sup> | No.<br>(n = 216) | FeNO,<br>ppb <sup>b</sup> | No.<br>(n = 52) | FeNO,<br>ppb <sup>b</sup> |
| Smoking status                 |                       |                           |                  |                           |                  |                           |                  |                           |                  |                           |                  |                           |                 |                           |
| Nonsmokers                     | 331                   | 18.4                      | 142              | 20.9                      | 83               | 18.4                      | 106              | 13.8                      | 189              | 16.0                      | 118              | 22.5                      | 24              | 14.9                      |
| Ex-smokers                     | 164                   | 17.5                      | 56               | 19.6                      | 35               | 19.0                      | 73               | 13.7                      | 108              | 15.6                      | 37               | 19.9                      | 19              | 17.4                      |
| Smokers                        | 159                   | 14.5                      | 70               | 15.9                      | 38               | 13.4                      | 51               | 12.5                      | 89               | 12.9                      | 61               | 15.9                      | 9               | 18.7                      |
| <i>P</i> for trend             | NA                    | .001                      | NA               | .01                       | NA               | .01                       | NA               | .2                        | NA               | .002                      | NA               | .002                      | NA              | .3                        |
| Daily tobacco consumption, g/d |                       |                           |                  |                           |                  |                           |                  |                           |                  |                           |                  |                           |                 |                           |
| None                           | 495                   | 18.1                      | 198              | 20.5                      | 118              | 18.5                      | 179              | 13.8                      | 297              | 15.9                      | 155              | 21.7                      | 43              | 15.8                      |
| <10                            | 84                    | 16.3                      | 42               | 17.5                      | 19               | 15.0                      | 23               | 14.5                      | 42               | 14.9                      | 35               | 18.2                      | 7               | 15.7                      |
| 10-20                          | 51                    | 13.7                      | 20               | 13.2                      | 16               | 12.0                      | 15               | 14.2                      | 31               | 13.1                      | 19               | 13.2                      | 1               | 19.4                      |
| >20                            | 23                    | 10.9                      | 8                | 15.1                      | 2                | 8.5                       | 13               | 8.5                       | 15               | 8.5                       | 7                | 12.7                      | 1               | 77.1                      |
| <i>P</i> for trend             | NA                    | <.001                     | NA               | .005                      | NA               | .0005                     | NA               | .007                      | NA               | <.001                     | NA               | .0004                     | NA              | .07                       |
| Pack-years                     |                       |                           |                  |                           |                  |                           |                  |                           |                  |                           |                  |                           |                 |                           |
| None                           | 331                   | 18.4                      | 142              | 20.9                      | 83               | 18.5                      | 106              | 13.8                      | 189              | 16.1                      | 118              | 22.6                      | 24              | 14.9                      |
| <10                            | 219                   | 16.5                      | 98               | 17.8                      | 53               | 17.7                      | 68               | 13.4                      | 121              | 15.2                      | 85               | 17.7                      | 13              | 19.3                      |
| 10-20                          | 58                    | 15.3                      | 19               | 17.2                      | 9                | 12.8                      | 30               | 13.2                      | 39               | 13.5                      | 8                | 17.3                      | 11              | 16.9                      |
| >20                            | 45                    | 14.1                      | 9                | 13.8                      | 10               | 11.5                      | 26               | 12.5                      | 36               | 12.4                      | 5                | 13.8                      | 4               | 15.7                      |
| <i>P</i> for trend             | NA                    | .0005                     | NA               | .02                       | NA               | .007                      | NA               | .3                        | NA               | .002                      | NA               | .006                      | NA              | .6                        |

Abbreviations: FeNO, fractional exhaled nitric oxide; NA, not applicable.

<sup>a</sup> FeNO values, given as geometric means, are adjusted for age, sex, height, center, and current asthma.<sup>b</sup> FeNO values, given as geometric means, are adjusted for age, sex, height, and center.

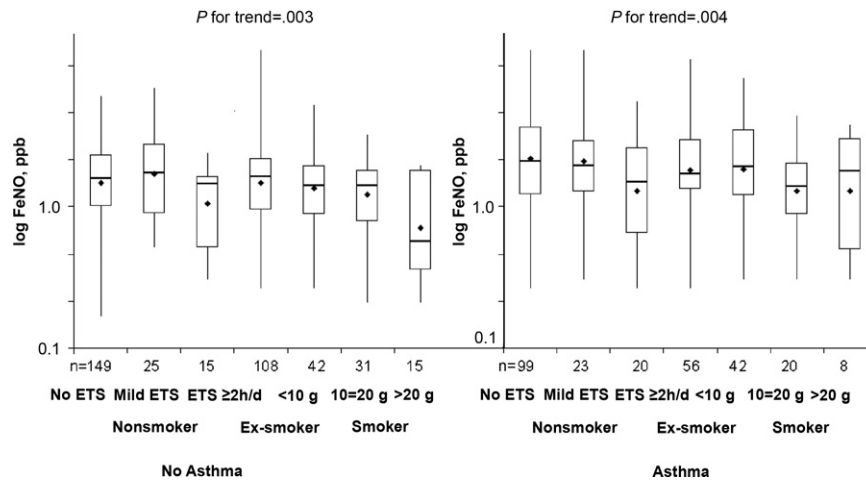


Figure 3. Effects of active and passive smoking on fractional exhaled nitric oxide (FeNO) measurements according to current asthma status. The horizontal line and the star in the middle of each box indicate the median and the mean, respectively. The top and bottom borders of each box mark the 75th and 25th percentiles, respectively. The whiskers above and below each box mark the maximum and minimum values. ETS indicates environmental tobacco smoke.

We confirmed the known positive associations between FeNO level and asthma, atopy, eosinophilia, and allergic rhinitis. We found a difference of borderline significance between men and women, an association previously found in other epidemiologic studies.<sup>4,5,21–24</sup> When sex and height were included in the regression models, sex,<sup>4,21,23</sup> height,<sup>5,25</sup> and sex and height<sup>24,26</sup> still remained significant. Heterogeneity of the factors taken into account in the analyses may partly explain the differences, and associations between FeNO and sex and height should be specifically considered in future epidemiologic studies.

Whether FeNO level is age dependent in adults is still a question in debate. Of the previous studies, some reported positive associations of FeNO level with age overall<sup>25</sup> or after stratification for smoking,<sup>22,24</sup> whereas others reported no association.<sup>21,23</sup> In the present study, we found no association of FeNO level with age even after stratification for smoking.

Table 3. Associations Between Active and Passive Smoking and FeNO Level According to Current Asthma Status (Results of Multivariate Analyses)<sup>a</sup>

|                        | No asthma (n = 386)              |         | Asthma (n = 268)                 |         |
|------------------------|----------------------------------|---------|----------------------------------|---------|
|                        | Estimate, mean (SE) <sup>a</sup> | P value | Estimate, mean (SE) <sup>a</sup> | P value |
| Nonsmoker, no/mild ETS | Reference                        |         | Reference                        |         |
| Nonsmoker, ETS ≥2 h/d  | −0.13 (0.06)                     | .03     | −0.18 (0.07)                     | .01     |
| Ex-smoker              | −0.02 (0.03)                     | .4      | −0.05 (0.05)                     | .3      |
| Smoker                 | −0.10 (0.03)                     | <.001   | −0.14 (0.04)                     | .02     |
| P for model            |                                  | .001    |                                  | .004    |

Abbreviations: ETS, environmental tobacco smoke; FeNO, fractional exhaled nitric oxide.

<sup>a</sup> Adjusted for age, sex, height, and center.

Heterogeneity of the study populations and of the factors taken into account in the analyses may explain the differences.

We extended the observations of the impact of active smoking on FeNO level by studying daily tobacco consumption and pack-years. We first confirmed the decrease in FeNO level with current active smoking, with ex-smokers having intermediate values between smokers and nonsmokers, as previously found.<sup>4,5,21,24</sup> The association was similar in men and women and according to asthma status. Furthermore, a significant decrease in FeNO level was found in nonsmokers exposed to ETS at least 2 h/d. This finding is consistent with that of the study by Yates et al,<sup>27</sup> in which FeNO levels in 15 controls decreased rapidly when exposed to cigarette smoke in a ventilated chamber compared with sham-exposed individuals. This finding is also coherent with that of a recent study<sup>10</sup> reporting a decrease in FeNO levels in 22 allergic asthmatic children with daily exposure to ETS compared with 48 unexposed asthmatic children.

Tiered explanations, each of biological plausibility, may explain the present results. Tobacco smoke contains a high concentration of NO, which exerts negative feedback on its synthesis,<sup>28</sup> and downregulation of NO synthase activity in cells of the respiratory tract by this mechanism has been suggested.<sup>29</sup> Observations of dose-effect relationships with daily tobacco consumption or pack-years are consistent with this negative feedback. Consistently, the expression of endothelial NO synthase in pulmonary arteries was reduced in smokers.<sup>30</sup> Marked effects of smoking in asthmatic or atopic individuals may be explained by the highest FeNO levels observed in nonsmokers in these subgroups and by the fact that it is easier to dip down when starting at high values. NO is a highly reactive molecule that can be oxidized or complexed with other biomolecules depending on the environment. Regarding the inflamma-

tory microenvironment that characterizes asthma,<sup>31</sup> with high levels of macrophages, neutrophils, and eosinophils, exaggerated production of NO in airways of asthmatic individuals in the presence of “oxidative stress” may produce highly reactive oxygen and nitrogen species.<sup>32</sup> FeNO was considered to better reflect processes that occur in relation to atopy rather than asthma. In atopic asthmatic individuals, high NO consumption, downregulation of NO synthase activity, and production of reactive species may coexist due to smoking, explaining the highest differences in FeNO levels between nonsmokers and current smokers observed in this subgroup.

In conclusion, besides active smoking, passive smoking is suggested as another factor that needs to be carefully considered in the study of FeNO levels in asthmatic adults. As suggested recently,<sup>2</sup> other steps in the progression of FeNO measurements from a research tool to a useful clinical test are necessary, and the use of FeNO level in the assessment of asthma should be discussed again, taking into account environmental factors, such as active and passive smoking, in the interpretation of FeNO values.

## ACKNOWLEDGMENTS

We thank all those who participated in the various aspects of this study: interviewers, technicians for lung function testing and skin prick tests, blood samplers, IgE determinators, and coders and those involved in quality control, data and sample management, and study supervision at all the centers. We are also grateful to the CIC-Inserm of Necker and Grenoble, which supported the study and in which participants were examined. We are indebted to all the individuals who participated, without whom this study would not have been possible. We thank Sofia Temam for technical help and Marie-Pierre Oryszczyn for helpful discussions.

The members of the EGEA Cooperative Group are as follows: *Coordination*: F. Kauffmann, F. Demenais (genetics), I. Pin (clinical aspects). *Respiratory epidemiology*: Inserm U 700, Paris: M. Korobaef (EGEA1), F. Neukirch (EGEA1); Inserm U 707, Paris: I. Annesi-Maesano; Inserm U 780, Villejuif: F. Kauffmann, N. Le Moual, R. Nadif, M. P. Oryszczyn; and Inserm U 823, Grenoble: V. Siroux. *Genetics*: Inserm U 393, Paris: J. Feingold; Inserm U 535, Villejuif: M. H. Dizier; Inserm U 946, Evry: E. Bouzigon, F. Demenais; and CNG, Evry: I. Gut, M. Lathrop. *Clinical centers*: Grenoble: I. Pin, C. Pison; Lyon: D. Ecochard (EGEA1), F. Gormand, Y. Pacheco; Marseille: D. Charpin (EGEA1), D. Vervloet; Montpellier: J. Bousquet; Paris Cochin: A. Lockhart (EGEA1), R. Matran (now in Lille); Paris Necker: E. Paty, P. Scheinmann; and Paris-Trousseau: A. Grimfeld, J. Just. *Data and quality management*: Inserm ex-U155 (EGEA1): J. Hochez; Inserm U 780, Villejuif: N. Le Moual, C. Ravault; Inserm U 946, Paris: N. Chateigner; and Grenoble: J. Ferran.

## REFERENCES

- de Jongste JC, Carraro S, Hop WC; CHARISM Study Group, Baraldi E. Daily telemonitoring of exhaled nitric oxide and symptoms in the treatment of childhood asthma. *Am J Respir Crit Care Med*. 2009;179:93–97.
- Pedersen S, O’Byrne PM. Exhaled nitric oxide in guideline-based asthma management. *Lancet*. 2008;372:1015–1017.
- Gibson PG. Using fractional exhaled nitric oxide to guide asthma therapy: design and methodological issues for ASthma TReatment ALgorithm studies. *Clin Exp Allergy*. 2009;39:478–490.
- Franklin PJ, Stick SM, Le Souef PN, Ayres JG, Turner SW. Measuring exhaled nitric oxide levels in adults: the importance of atopy and airway responsiveness. *Chest*. 2004;126:1540–1545.
- Olin A-C, Rosengren A, Thelle DS, Lissner L, Bake B, Toren K. Height, age, and atopy are associated with fraction of exhaled nitric oxide in a large adult general population sample. *Chest*. 2006;130:1319–1325.
- Malinovschi A, Janson C, Hogman M, et al. Both allergic and nonallergic asthma are associated with increased FE(NO) levels, but only in never-smokers. *Allergy*. 2009;64:55–61.
- Michils A, Louis R, Peche R, Baldassarre S, Van Muylem A. Exhaled nitric oxide as a marker of asthma control in smoking patients. *Eur Respir J*. 2009;33:1295–1301.
- Bessa V, Tseliou E, Bakakos P, Loukides S. Noninvasive evaluation of airway inflammation in asthmatic patients who smoke: implications for application in clinical practice. *Ann Allergy Asthma Immunol*. 2008;101:226–232.
- Gabriele C, Asgarali R, Jaddoe VW, Hofman A, Moll HA, de Jongste JC. Smoke exposure, airway symptoms and exhaled nitric oxide in infants: the Generation R study. *Eur Respir J*. 2008;32:307–313.
- Laoudi Y, Nikasinovic L, Sahraoui F, Grimfeld A, Momas I, Just J. Passive smoking is a major determinant of exhaled nitric oxide levels in allergic asthmatic children. *Allergy*. 2009; Epub ahead of print.
- Kauffmann F, Dizier MH, Annesi-Maesano I, et al. EGEA (Epidemiological study on the Genetics and Environment of Asthma, bronchial hyperresponsiveness and atopy): descriptive characteristics. *Clin Exp Allergy*. 1999;29(suppl 4):17–21.
- Kauffmann F, Dizier M-H, Pin I, et al. Epidemiological study of the genetics and environment of asthma, bronchial hyperresponsiveness, and atopy: phenotype issues. *Am J Respir Crit Care Med*. 1997;156:1235–1295.
- Siroux V, Boudier A, Bousquet J, et al. Phenotypic determinants of uncontrolled asthma. *J Allergy Clin Immunol*. 2009;124:681–687.
- Global Initiative for Asthma. *Global Strategy for Asthma Management and Prevention*. Bethesda, MD: National Heart, Lung, and Blood Institute; 1995 (updated 2008). NIH publication 95-3659.
- ATS/ERS recommendations for standardized procedures for the on-line and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide. *Am J Respir Crit Care Med*. 2005;171:912–930.
- Liang KY, Zeger SL. Regression analysis for correlated data. *Annu Rev Public Health*. 1993;14:43–68.
- Siroux V, Guilbert P, Le Moual N, Oryszczyn MP, Kauffmann F. Influence of asthma on the validity of reported lifelong environmental tobacco smoke in the EGEA study. *Eur J Epidemiol*. 2004;19:841–849.
- Deykin A, Massaro AF, Drazen JM, Israel E. Exhaled nitric oxide as a diagnostic test for asthma: online versus offline techniques and effect of flow rate. *Am J Respir Crit Care Med*. 2002;165:1597–1601.
- Borrill Z, Clough D, Truman N, Morris J, Langley S, Singh D. A comparison of exhaled nitric oxide measurements performed using three different analysers. *Respir Med*. 2006;100:1392–1396.
- Bouzigon E, Bechet M, Monier F, et al. Interactive effects of NOS1 and NOS3 genetic variants and asthma on FeNO level in the EGEA study. *Eur Respir J*. 2009;34:161s.
- Levesque MC, Hauswirth DW, Mervin-Blake S, et al. Determinants of exhaled nitric oxide levels in healthy, nonsmoking African American adults. *J Allergy Clin Immunol*. 2008;121:396–402.

- 
22. Sundry JS, Hauswirth DW, Mervin-Blake S, et al. Smoking is associated with an age-related decline in exhaled nitric oxide. *Eur Respir J*. 2007; 30:1074–1081.
23. Travers J, Marsh S, Aldington S, et al. Reference ranges for exhaled nitric oxide derived from a random community survey of adults. *Am J Respir Crit Care Med*. 2007;176:238–242.
24. Smit LA, Heederik D, Doekes G, Wouters IM. Exhaled nitric oxide in endotoxin-exposed adults: effect modification by smoking and atopy. *Occup Environ Med*. 2009;66:251–255.
25. Olin A-C, Bake B, Toren K. Fraction of exhaled nitric oxide at 50 mL/s: reference values for adult lifelong never-smokers. *Chest*. 2007;131: 1852–1856.
26. Dressel H, de la Motte D, Reichert J, et al. Exhaled nitric oxide: independent effects of atopy, smoking, respiratory tract infection, gender and height. *Respir Med*. 2008;102:962–969.
27. Yates DH, Breen H, Thomas PS. Passive smoke inhalation decreases exhaled nitric oxide in normal subjects. *Am J Respir Crit Care Med*. 2001;164:1043–1046.
28. Buga GM, Griscavage JM, Rogers NE, Ignarro LJ. Negative feedback regulation of endothelial cell function by nitric oxide. *Circ Res*. 1993; 73:808–812.
29. Kharitonov SA, Robbins RA, Yates D, Keatings V, Barnes PJ. Acute and chronic effects of cigarette smoking on exhaled nitric oxide. *Am J Respir Crit Care Med*. 1995;152:609–612.
30. Barbera JA, Peinado VI, Santos S, Ramirez J, Roca J, Rodriguez-Roisin R. Reduced expression of endothelial nitric oxide synthase in pulmonary arteries of smokers. *Am J Respir Crit Care Med*. 2001;164:709–713.
31. Ricciardolo FL, Di Stefano A, Sabatini F, Folkerts G. Reactive nitrogen species in the respiratory tract. *Eur J Pharmacol*. 2006;533:240–252.
32. Andreadis AA, Hazen SL, Comhair SA, Erzurum SC. Oxidative and nitrosative events in asthma. *Free Radic Biol Med*. 2003;35:213–225.
- Requests for reprints should be addressed to:*  
*Rachel Nadif, PhD*  
*Respiratory and Environmental Epidemiology*  
*CESP Centre for Research in Epidemiology and Population Health*  
*U1018, Inserm*  
*F-94807, Villejuif, France*  
*E-mail: [rachel.nadif@inserm.fr](mailto:rachel.nadif@inserm.fr)*
-