Respiratory Medicine (2008) **102**, 962–969

ELSEVIER

respiratoryMEDICINE

Exhaled nitric oxide: Independent effects of atopy, smoking, respiratory tract infection, gender and height

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Received 8 November 2007; accepted 20 February 2008
Available online 18 April 2008

KEYWORDS
Exhaled nitric oxide;
Respiratory allergy;
Smoking;
Respiratory tract infection;
Rhinitis;
Asthma;
Multivariate analysis

Summary
Measurement of exhaled nitric oxide is widely used in respiratory research and clinical practice, especially in patients with asthma. However, interpretation is often difficult, due to common interfering factors, and little is known about interactions between factors. We assessed the influences and interactions of factors such as smoking, respiratory tract infections and respiratory allergy concerning exhaled nitric oxide values, with the aim to derive a scheme for adjustment. We studied 897 subjects (514 females, 383 males; mean age $\pm$ standard deviation 34.5 ± 13.0 years) with and without respiratory allergy (allergic rhinitis and/or asthma), smoking and respiratory tract infection. Logarithmic nitric oxide levels were described by an additive model comprising respiratory allergy, smoking, respiratory tract infection, gender and height ($p \leqslant 0.001$ each), without significant interaction terms. Geometric mean was 17.5 ppb in a healthy female non smoker of height 170 cm, whereby respiratory allergy corresponded to a change by factor 1.50, smoking 0.63, infection 1.24, male gender 1.17, and each 10 cm increase (decrease) in height to 1.11 (0.90). Factors were virtually identical when excluding asthma and using the category allergic rhinitis instead of respiratory allergy ($n = 863$). Within each category formed by combinations of these different predictors, the range of residual variation was approximately constant. We conclude that the factors influencing exhaled nitric oxide, which we analyzed, act independently of each other. Thus, circumstances such as smoking

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0954-6111/$ - see front matter
doi:10.1016/j.rmed.2008.02.012

and respiratory tract infection do not appear to affect the usefulness of exhaled nitric oxide, provided that appropriate factors for adjustment are applied.

Introduction

A multitude of studies have demonstrated the usefulness of measuring the fraction of exhaled nitric oxide (FE_{NO}) in patients with asthma,[1] allergic rhinitis, or atopy,[2] including the evaluation of treatment effects.[3] Besides disease and therapy,[4] there are further factors influencing FE_{NO}.[5] Some of these, such as performing spirometry prior to FE_{NO} measurement,[6] can be easily taken into account in clinical practice, while this is more difficult for others, such as current smoking or respiratory tract infections, which are difficult to exclude in patients visiting a physician.

Smoking leads to a decrease in FE_{NO},[7] though values are still higher in smokers with asthma than in those without.[8] The effects of smoking have been further specified by revealing that the logarithm of FE_{NO} showed additive contributions from smoking history and the hours since the last cigarette.[9] FE_{NO} is also known to be elevated in upper respiratory tract infections,[10] and correspondingly in exacerbations of patients with chronic obstructive pulmonary disease (COPD)[11] or in pulmonary infections of lung transplant patients.[12] Studies furthermore demonstrated an association with height[13] and gender[14]; the latter, however, might be attributable to differences in height.[13] In addition, age might be associated with FE_{NO},[13] especially in children[15] and very old subjects showing elevated values.[16]

While clearly establishing the influences on FE_{NO}, the available results are not easily applicable for the evaluation of individual values in clinical practice. Some studies were restricted to healthy subjects with and without atopy,[14,15,17] and others[2,13] described the multiple effects with great scrutiny in large populations but the complex results did not translate into easily applicable schemes. Only one study[18] based on a large random sample reported reference ranges for combinations of factors as derived by multivariate analysis. In addition, most studies did not include respiratory tract infections into the set of multiple potential influence factors and the issue of interactions with this factor remains unresolved. Correspondingly, a common conclusion seems to be, e.g., that FE_{NO} is difficult to interpret in smokers or subjects suffering from respiratory tract infections, and FE_{NO} is often not assessed in these.

Therefore, an analysis of factors affecting FE_{NO} that aims to meet the practical need for simple, transparent, broadly-to-apply reference values could be a step forward. On the statistical side this involves as a major question, whether the various factors show multiple interactions, including different ranges of variation in different strata, or whether they act independently of each other, with similar variation. The latter would offer the potential to derive a reference state with easily applicable factors for adjustment and variability. It would thus probably facilitate a sensible use of FE_{NO} measurements across populations. Consequently, the aim of the present study was to quantify the single and combined effects and interactions of factors such as respiratory allergy, smoking, respiratory tract infection, height, lung function, age and gender on FE_{NO}, with special focus on the attempt to express the results in a form that favored their application in clinical practice.

Methods

Study population

Measurements were performed during pre-employment examinations and occupational preventive medical checkups in the Outpatient Clinic for Occupational and Environmental Medicine of the Ludwig-Maximilians-University, Munich. Consecutive examinations ($n = 1037$) between April 2005 and March 2007 were examined for analysis. Subjects using oral or inhaled corticosteroids, or showing airways diseases other than asthma and rhinitis or with incomplete data were excluded from the analysis. In case of repeated measurements the first one was taken. After applying these criteria 897 subjects were included (Table 1). This population comprised 34 subjects with mild allergic asthma, having either no or only bronchodilator therapy. Analyses were performed either with or without these subjects.

Table 1 Characteristics of the study population.

n	897
Female, *n* (%)	514 (57.3)
Age, yr	34.5±13.0
Height, cm	172.0±8.8
Weight, kg	71.4±15.7
BMI, m/kg^2	24.0±4.3
FE_{NO}, ppb*	19.6÷1.92
FEV_1, %predicted	106.6±13.4
FVC, %predicted	110.3±13.3
FEV_1/VC, %predicted	98.3±7.9
Respiratory allergies (rhinitis and/or asthma), *n* (%)	206 (23.0)
Allergic rhinitis, *n* (%)	193 (21.5)
Allergic asthma, *n* (%)	34 (3.8)
Current smoking, *n* (%)	218 (24.3)
Respiratory tract infection, *n* (%)	190 (21.2)

Data are presented either as the number of subjects (% in parentheses) or as mean±SD. BMI = body mass index, FE_{NO} = fractional concentration of exhaled nitric oxide, FEV_1 = forced expiratory volume in 1 s, FVC = forced vital capacity.

*Due to the data distribution geometric mean and SD are given, the latter being expressed as a factor indicated by ÷. The geometric mean has to be multiplied with and divided by this SD factor.

Assessments

FE_{NO} was determined during a single exhalation using a chemiluminescence analyzer (NOA 280^{TM}, Sievers, Boulder, Co, USA) according to international guidelines.[5] After inhaling ambient air subjects started to expire through a mouthpiece against a positive pressure, aiming to achieve a flow rate of 50 mL/s under visual control on a computer screen. Ambient air levels normally showed NO levels <15 ppb and a correlation analysis showed no relationship between FE_{NO} and ambient air NO. Measurements were performed at least in triplicate. The mean of three reproducible values was taken for analysis. Acceptable measurements had to show a clearly identifiable plateau (within 10% of each other, typically $<5\%$) and a flow rate within 10% (typically $<5\%$) of the target rate during the plateau measurement. The analyzer was calibrated regularly using a certified calibration gas (Linde AG, Munich, Germany).

Afterwards, inspiratory vital capacity (VC) and forced expiratory volume in 1 s (FEV_1) were determined (MasterLab^{TM}, Jaeger, Germany) following the established guidelines.[19] At least three technical acceptable flow-volume maneuvres were performed and the highest values were taken. The presence of respiratory allergies (allergic rhinitis and/or asthma; yes/no), smoking during the last 4 weeks (yes/no) and respiratory tract infection during the last 4 weeks (yes/no) was assessed by a physician when taking the subjects' history. The study was approved by the Ethical Review Board of the LMU Munich.

Data analysis

In accordance with the usual approach, values of FE_{NO} were $\log_{10}$-transformed for all statistical analyses to achieve their normal distribution. The log-transformed values were used to derive geometric mean values and standard deviations (SD) of FE_{NO}. Geometric SD was expressed as a dimensionless factor with regard to the geometric mean value, and this specific relation was indicated by the symbol $\div$. The $\log_{10}$ FE_{NO} values were also used as dependent variables in analyses of covariance (ANCOVA), which represents a combination of standard analysis of variance (ANOVA) and multilinear regression analysis. The linear covariates used were age, height, weight, body mass index (BMI), FEV_1 and VC. The dichotomous variables were gender, respiratory allergy, smoking and respiratory tract infection. The model was computed either including or excluding subjects with asthma ($n = 34$), corresponding to either the category respiratory allergy (when including asthma) or allergic rhinitis (when excluding asthma).

The basic ANCOVA model incorporated not only the linear superposition of all main factors but also all interactions. Based on the p-values regarding the different predictors and interaction terms and by eliminating the term with the greatest p-value in a step-down fashion, a minimal model was derived. In case of correlated covariates the validity of the approach was checked by alternative choices of covariates to avoid potentially misleading correlations. As all interactions turned out to be non-significant, the final minimal model provided estimates of the four remaining main effects (categories) and one linear regression coefficient (see results). As it could have been possible that the regression coefficient took different values in the subgroups formed by the categories, additional linear regression analyses were performed within these subgroups.

The approach described resulted in a standard additive ANCOVA model for $\log_{10} FE_{NO}$. As addition of log values corresponds to multiplication of non-log values, this led to a multiplicative model regarding FE_{NO}. To obtain the FE_{NO} model we retransformed all ANCOVA terms using the antilog. Thus, the additive terms in the model for $\log_{10} FE_{NO}$ became multiplicative terms in the model for FE_{NO}. These multiplicative terms represent the effects of different factors on FE_{NO} relative to a reference value.

The result was also given as an explicit formula comprising all factors of influence relative to the geometric mean FE_{NO} of a reference state. To facilitate the practical application, we additionally computed tables of FE_{NO} stratified according to gender and height. These tables were adjusted for the remaining (categorical) predictors, whose effects can be taken into account by the multiplicative factors reported. The p-values <0.05 were considered statistically significant. Statistical calculations were performed using SPSS 14.0 (SPSS Inc., Chicago, IL).

Results

Table 1 shows the characteristics of the study population. Geometric mean ($\div$SD) FE_{NO} was $19.6 \div 1.92$ ppb in the whole study population. Univariate analyses (t-test) showed FE_{NO} to be lower ($p<0.001$ each) in females ($17.4 \div 1.91$ vs. $23.0 \div 1.86$ ppb), subjects without respiratory allergies ($17.8 \div 1.78$ vs. $27.3 \div 2.16$ ppb), smokers ($13.5 \div 1.98$ vs. $22.1 \div 1.81$ ppb) and subjects without respiratory tract infection ($18.5 \div 1.88$ vs. $24.1 \div 1.99$ ppb) as compared to their respective counterparts. Subjects with allergic rhinitis and asthma showed higher values than those with allergic rhinitis without asthma ($42.1 \div 2.2$ vs. $26.4 \div 2.1$ ppb; $p = 0.008$).

The final ANCOVA models did not comprise any interactions, as these were statistically nonsignificant ($p \geqslant 0.23$ each). Respiratory allergy, smoking, respiratory tract infection and gender remained as categorical factors and height as a covariate ($p \leqslant 0.001$ each) in the model comprising subjects with asthma; Table 2 provides the coefficients relating the predictors to $\log_{10} FE_{NO}$. The explained variance

Table 2 Coefficients of $\log_{10} FE_{NO}$ with 95% confidence intervals (95%CI) as obtained in 897 subjects in the final ANCOVA model comprising subjects with asthma.

Variable	Coefficient (95%CI)
Intercept	0.453 (0.061; 0.846)
Male gender	0.070 (0.028; 0.111)
Respiratory allergy	0.175 (0.136; 0.213)
Smoking	−0.203 (−0.240; −0.165)
Respiratory tract infection	0.092 (0.052; 0.131)
Height per 10 cm (regression coefficient)	0.0464 (0.0230; 0.0700)

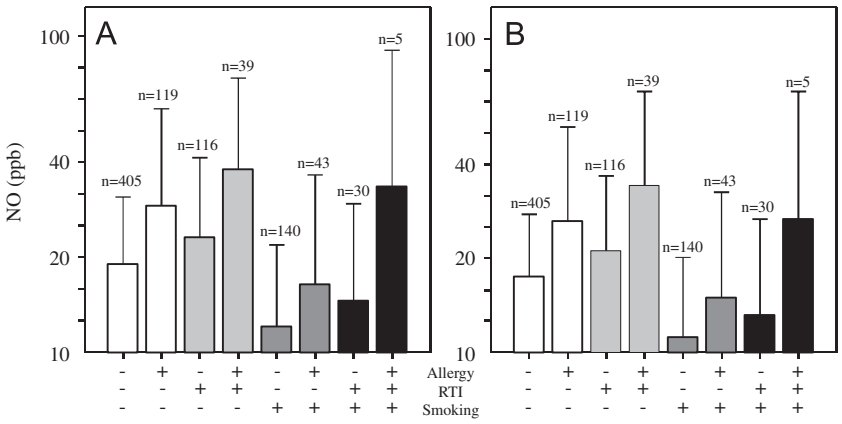

Figure 1 Mean and standard deviation (SD) of FE_{NO} (log scale) in the subgroups formed by different combinations of the variables smoking, respiratory tract infection (RTI) and respiratory allergy ($n = 897$). Panel A shows the measured data in the sample, panel B the data adjusted to female gender and a height of 170 cm. The graph illustrates that the different variables of influence were additive with regard to the mean (expected) value of FE_{NO}. This can be seen e.g. within the left four bars where the presence of an RTI in non-smokers results in the same upward shift of FE_{NO} for allergic and non-allergic subjects, i.e., RTI acts homogenously and independent from allergy. The same is true when recognizing that e.g. for smoking in panel B the pattern of the left four bars is very similar to that of the right four bars but lowered by a certain amount which represents the effect of smoking acting homogeneously on the groups formed by the combinations of the two variables, allergy and RTI.

(R^2) was 25.7% of the total variance. Figure 1A illustrates FE_{NO} in the eight subgroups formed by the possible combinations of allergy, smoking and infection. Virtually the same picture, though on a lower overall level, emerged when values were adjusted to female gender and normalized to height 170 cm (Figure 1B).

To test for the homogeneity of the relation between FE_{NO} and height as derived from ANCOVA, we performed additional linear regression analyses within each of the two subgroups formed by each categorical predictor. All regression coefficients regarding height were different from zero ($p<0.02$ each), and all of their 95% confidence intervals were overlapping. Based on these results, a single additive model that comprised no interactions between the categorical variables and no interaction betweeen the linear covariate height and the categorical variables described $\log_{10}$ FE_{NO} adequately. After retransformation of parameter values from the log into the original FE_{NO} domain, the corresponding formula for the expected value of FE_{NO} was

$$FE_{NO}(\text{ppb}) = 17.49 \times \left\{ \begin{matrix} \text{allergy} \\ 1.496 \\ \text{yes} \end{matrix} \right\} \times \left\{ \begin{matrix} \text{smoking} \\ 0.627 \\ \text{yes} \end{matrix} \right\} \times \left\{ \begin{matrix} \text{infection} \\ 1.235 \\ \text{yes} \end{matrix} \right\} \times \left\{ \begin{matrix} \text{male} \\ 1.174 \\ \text{yes} \end{matrix} \right\} \times 1.113^{(\text{height(cm)}-170)/10}.$$

When the ANCOVA analysis was restricted to patients with allergic rhinitis without asthma, the results were virtually the same. To illustrate the application of these formulae, Table 3 provides geometric mean values of FE_{NO} for females over a height range from 160 to 185 cm in intervals of 5 cm, and for males over a height range from 165 to 190 cm in the population including subjects with allergic asthma.

As a next step, we compared variances within the subgroups formed by the possible combinations of allergy, smoking and infection. These variances were significantly different from each other (Levene test, $p<0.001$); however, SD values within the different subgroups (see Figure 1) differed from the adjusted pooled SD by not more than −24% and 22%. For practical purposes we thus considered it justified to take the pooled variance of adjusted values as the variation within each of the subgroups. To describe the range of variation within each of the subgroups defined by combination of the categorical predictors, the corresponding expected value of FE_{NO} (see formula or Table 3) can be multiplied either by the factor respresenting the geometric pooled adjusted SD or by factors representing percentiles. Following the arguments given above, these factors can be taken as approximately the same for all subgroups. The respective adjusted SD factor was 1.753, while the 5th and the 95th percentile factors were 0.421 and 2.44, respectively, and the 10th and 90th percentile factors 0.502 and 1.97.

Discussion

The present study addressed the combined effects and potential interactions of factors that are known to affect the level of FE_{NO}. The factors studied covered basic anthropometric characteristics as well as circumstances often encountered in patients visiting an outpatient clinic. As a result, the statistically significant predictors which comprised respiratory allergy or allergic rhinitis, smoking, respiratory tract infection, gender and height were found to have quite homogeneous effects on FE_{NO}. The homogeneity was reflected in an additive model of log FE_{NO}, containing only main factors without signs of statistical interaction. Upon retransformation of the logarithm into the original FE_{NO} domain, the model translated into a simple multiplicative scheme to adjust for various effects on FE_{NO}.

Regarding the kind of influences on FE_{NO}, our findings were in accordance with published data. FE_{NO} is known to be elevated in subjects with atopy without symptoms,[20] or allergic rhinitis,[2] or asthma.[1] The variable respiratory

Table 3 Examples of expected geometric mean values of FE_{NO} (ppb) in females and males as derived from the formula given in the results.

Females								
Smoking	–	–	–	–	+	+	+	+
Infection	–	–	+	+	–	–	+	+
Allergy	–	+	–	+	–	+	–	+
Height (cm)								
160	15.7	23.5	19.4	29.0	9.9	14.7	12.2	18.2
165	16.6	24.8	20.5	30.6	10.4	15.5	12.8	19.2
170	17.5	26.2	21.6	32.3	11.0	16.4	13.5	20.2
175	18.5	27.6	22.8	34.1	11.6	17.3	14.3	21.4
180	19.5	29.1	24.0	35.9	12.2	18.2	15.1	22.5
185	20.5	30.7	25.4	37.9	12.9	19.3	15.9	23.8
Males								
Smoking	–	–	–	–	+	+	+	+
Infection	–	–	+	+	–	–	+	+
Allergy	–	+	–	+	–	+	–	+
Height (cm)								
165	19.5	29.1	24.0	35.9	12.2	18.2	15.1	22.5
170	20.5	30.7	25.3	37.9	12.9	19.2	15.9	23.8
175	21.7	32.4	26.7	40.0	13.6	20.3	16.8	25.1
180	22.8	34.2	28.2	42.2	14.3	21.4	17.7	26.4
185	24.1	36.1	29.8	44.5	15.1	22.6	18.7	27.9
190	25.4	38.0	31.4	47.0	15.9	23.8	19.7	29.4

To estimate the variation within each of the categories, the FE_{NO} value has to be multiplied with the 5th or 10th or 90th or 95th percentile factors (0.42, 0.50, 1.97, and 2.44, respectively).

allergy used by us included allergic rhinitis and/or asthma as reported by the subjects. We did not additionally validate the answers through objective markers of atopy or assessment of bronchial hyperreactivity; thus, we cannot rule out a bias concerning this variable. The questions we used are standard tools to assess allergic rhinitis and asthma in large international surveys.[21] If there should have been a bias, it possibly has favored negative answers, as subjects may have symptoms but are not aware of their diagnosis, leading to underestimation of the effects of atopy on FE_{NO}. However, the factor which we found was even greater than those reported previously by using objective markers.[13,18]

The number of subjects reporting asthma was low ($n = 34$), not allowing for further stratification. We only included subjects without steroid medication, i.e., presumably mild asthma. These subjects accounted for 3.8% of the study population, in accordance with the expected prevalence in the general population. It seemed reasonable to keep these subjects in the analysis as lung function was normal and FE_{NO} values were not biased by corticosteroids. The combination of self-reported allergic rhinitis and/or asthma into a single variable could be considered questionable. We would like to note, however, that the statistical model of $\log FE_{NO}$ remained essentially unchanged, when using the category of allergic rhinitis and restricting the analysis to subjects without asthma.

We did not document whether the allergic rhinitis was persistent or seasonal and also did not have reliable information whether subjects with seasonal allergies were examined in their respective season. This might be of interest as FE_{NO} values, which are on average lower in allergic rhinitis compared to asthma[22] can reach similar levels after allergen exposure.[23] Since under the conditions of clinical practice acute allergen exposure seems to be difficult to assess with reliability, we focused on the mere presence of a clinically apparent respiratory tract allergy, neglecting the exposure status. Using this approach the multiplicative adjustment of FE_{NO} for allergy was found to be about 1.50, being similar in all subgroups. From the average effect size reported by Olin et al.,[13] an average factor of 1.23 across the population studied can be derived, and in another study[18] this value was 1.19. The higher factor found in the present study might be well explained by the fact that subjects were required to report not the presence of elevated IgE or positive skin prick test but the occurrence of clinical symptoms, at present or in the past. Most importantly for practical purposes, our results proved that a single factor of FE_{NO} with regard to respiratory allergy could be applied independently from other influences on FE_{NO}.

A second major influence on FE_{NO} is exerted by smoking, which has been consistently shown to reduce FE_{NO} levels, although the effect has not often been studied using multivariate models. We used only the information on current smoking, as this is primarily available in clinical practice. Smokers with asthma have been shown to exhibit a short-term increase of FE_{NO} after smoking, with a half-life of about 2.4 h, that in the log domain was additive to the long-term effect,[7] while in smokers without asthma FE_{NO} was elevated 1 and 10 min after inhalation of a cigarette, with return to the baseline within 20 min.[24] In our setting, the time until measurement after the subject's arrival was

$\geqslant$30 min, rendering a potential acute effect of smoking on FE_{NO} unlikely.

The picture emerging from the literature with regard to daily or cumulative cigarette consumption is not fully consistent. FE_{NO} has been reported to differ between never- and ex-smokers, after adjustment for gender, age, height, IgE sensitization and FEV_1.[25] In contrast, within a large population-based study, also stratifying by gender and atopy, there was no such difference.[13] Moreover, in subjects quitting smoking FE_{NO} is known to increase to about the level measured in normal nonsmokers within 1–8 weeks of abstinence.[26,27] We considered these findings as a justification to subsume ex- and never-smokers into the same category. Stratification of ex-smokers according to the time since smoking cessation and pack years would also have stressed the limits of multivariate analysis capabilities in our sample. Probably these factors play a role mainly in the presence of clinically relevant COPD (GOLD$\geqslant$1) which we excluded. The available data suggest that a decrease in FE_{NO} by about factor 0.84 for each 10 pack years can occur, but only in subjects with asthma.[9] As these subjects represented only a small proportion of our sample, we considered the cumulative smoking history as a minor issue regarding FE_{NO}.

While there are considerable data demonstrating the effect of smoking on FE_{NO}, the appropriate adjustment has been rarely studied using log NO analysis. In comprehensive multivariate models comparing smokers with never- and ex-smokers, the overall factor of FE_{NO} corresponding to smoking was 0.7 in one study,[13] and 0.8 for smokers compared to non-smokers in another,[18] similar to the value of 0.63 we found. FE_{NO} is also known to be decreased in smokers compared to non-smokers, if subjects have asthma.[8,28] Moreover, the mean values reported in a comprehensive study[13] and stratified according to atopy allow us to derive factors for current smoking vs. non-smoking of about 0.70 in atopic and 0.63 in nonatopic subjects. One of the novel findings of our study was the explicit proof of a similar effect of smoking in all subgroups including those stratified by respiratory allergy, respiratory tract infections and gender, as demonstrated by the absence of interaction terms and illustrated by the parallel shift in Figure 1. This allows the use of a single multiplicative factor to adjust for smoking independently from other influencing variables, which seems to be particularly useful for clinical practice.

In our study population the presence of respiratory tract infections during the last 4 weeks was associated with elevated FE_{NO} values. The respective factor was 1.24, again similar in all subgroups and without interactions. The infections may have included a variety of locations and viral or bacterial pathogens. Rhinovirus infection has been shown to raise FE_{NO},[29,30] and 1–2 days after the onset of symptoms normal subjects with symptomatic upper respiratory tract infections showed increased FE_{NO}.[10] Three weeks later FE_{NO} was back to normal. Our subjects were asked for respiratory tract infections during the last 4 weeks, as we wanted to keep the time window large enough to exclude potential effects on the values used as reference. Although the effect of infection might have been underestimated, it was still considerable. The corresponding increase was smaller than that indicated by another study,[10] which, however, at that time used a method greatly affected by nasal NO. Unfortunately, our findings cannot be compared with those of the large population-based studies, as these did not report a variable for respiratory tract infection.[13,18] Our findings indicate that respiratory tract infections should be taken into account regarding FE_{NO} and that this can be easily done by a constant multiplicative factor.

Both gender and height turned out to be independently associated with FE_{NO}, corresponding to a factor of 1.17 for male vs. female gender and of 1.11 per 10 cm increase in height. Gender has been reported as a determinant of FE_{NO} in adults,[14] while there was no association with height. Multivariate analysis of data from a community survey derived a 1.26 times higher FE_{NO} in males compared to females.[18] However, there are also data indicating that the association between FE_{NO} and gender is explained by differences in height.[13,17] Remarkably, the average factor relating FE_{NO} to height as derivable from these data was about 1.14 and 1.09 per 10 cm increase and thus very similar to the coefficient found by us. Height was found to be the major determinant of FE_{NO} also in healthy children, in contrast to gender.[31] Our data indicate that in adults both gender and height are relevant for FE_{NO} and that their effect is independent of that of other predictors.

An increase of FE_{NO} with age has been found,[13,17] in the range of 1.09 per 10-year increase as can be derived from these data, while other results did not indicate such a dependence.[14,18] Moreover, increases of FE_{NO} with age have been reported in children,[15] as well as elevated values in very old subjects.[16] We did not observe an association of FE_{NO} with age in our study population. This might have been partially due to the low average age and the small number of older subjects. Irrespective of this, the clear-cut and robust effect of height demonstrated that the power to identify independent covariates in the range of subjects studied was high.

Considering the potential practical implications of our findings, it should be noted that there was still great residual variation within the different strata. This is illustrated by the ratios between standard deviations and differences between groups (Figure 1). It is an open question whether there are other easy-to-assess predictors that could markedly reduce the observed residual variation, which seems to reflect the multifactorial character of FE_{NO}. Other measures related to airway inflammation, such as sputum eosinophilia or bronchial hyperreactivity, are known to show correlations with FE_{NO},[32] which are independent from confounders like height and gender.[33] Based on the fact that variation was similar across strata, we propose a single set of factors for describing the variation. This greatly simplifies their use. The factors representing the 10th and 90th percentiles turned out to be about 2, and those representing the 5th and 95th percentiles about 2.5, again user friendly. These factors will allow location of a patient's FE_{NO} within the respective stratum for all practical purposes sufficiently well.

There are, however, limitations to be considered. The different strata were occupied by unequal numbers of subjects, although this did not grossly affect our analysis. Figure 1 indicates that shifts were close to parallel, the largest one occurring in the group of smokers with respiratory tract infection and respiratory allergy which

comprised only five subjects. FE_{NO} can be used sensibly for longitudinal follow-up in the same individuals.[3] In contrast, single measurements of absolute levels must not be overstressed, due to the broad range of variation. Moreover, calibration errors and gas stability are practically relevant issues, despite the fact that commercial analyzers seem to yield comparable readings.[34] However, another study found significantly different FE_{NO} values when comparing different FE_{NO} analyzers.[35] These findings have an impact on absolute values of FE_{NO} by altering the basic reference value assumed, but not on our result of an independent superposition of different factors of influence on FE_{NO}.

In conclusion, the results of the present study suggested the use of independent multiplicative factors to adjust FE_{NO} for the influence of the predictors respiratory allergy, smoking, respiratory tract infection, gender and height. Moreover, the distribution of data within the strata formed by the predictors could be described by a single set of multiplicative factors. Qualitatively and quantitatively, our findings fit well those of published studies involving comprehensive, detailed analyses. In addition to these, they offer an easily applicable scheme to adjust for patients' characteristics and circumstances that are common in real-life clinical practice.

Conflict of interest statement

All authors have no conflict of interest.

References

1. Alving K, Weitzberg E, Lundberg JM. Increased amount of nitric oxide in exhaled air of asthmatics. *Eur Respir J* 1993;**6**:1368–70.
2. Olin AC, Alving K, Toren K. Exhaled nitric oxide: relation to sensitization and respiratory symptoms. *Clin Exp Allergy* 2004;**34**:221–6.
3. Silkoff PE, McClean P, Spino M, Erlich L, Slutsky AS, Zamel N. Dose–response relationship and reproducibility of the fall in exhaled nitric oxide after inhaled beclomethasone dipropionate therapy in asthma patients. *Chest* 2001;**119**:1322–8.
4. Taylor DR, Pijnenburg MW, Smith AD, De Jongste JC. Exhaled nitric oxide measurements: clinical application and interpretation. *Thorax* 2006;**61**:817–27.
5. ATS/ERS recommendations for standardized procedures for online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide. *Am J Respir Crit Care Med* 2005;**171**:912–30.
6. Gabriele C, Pijnenburg MWH, Monti F, Hop W, Bakker ME, de Jongste JC. The effect of spirometry and exercise on exhaled nitric oxide in asthmatic children. *Pediatr Allergy Immunol* 2005;**16**(243):247.
7. 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**:609–12.
8. Horvath I, Donnelly LE, Kiss A, Balint B, Kharitonov SA, Barnes PJ. Exhaled nitric oxide and hydrogen peroxide concentrations in asthmatic smokers. *Respiration* 2004;**71**:463–8.
9. McSharry CP, McKay IC, Chaudhuri R, Livingston E, Fraser I, Thomson NC. Short and long-term effects of cigarette smoking independently influence exhaled nitric oxide concentration in asthma. *J Allergy Clin Immunol* 2005;**116**:88–93.
10. Kharitonov SA, Yates D, Barnes PJ. Increased nitric oxide in exhaled air of normal human subjects with upper respiratory tract infections. *Eur Respir J* 1995;**8**:295–7.
11. Bhowmik A, Seemungal TA, Donaldson GC, Wedzicha JA. Effects of exacerbations and seasonality on exhaled nitric oxide in COPD. *Eur Respir J* 2005;**26**:1009–15.
12. Antus B, Csiszer E, Czebe K, Horvath I. Pulmonary infections increase exhaled nitric oxide in lung transplant recipients: a longitudinal study. *Clin Transplant* 2005;**19**:377–82.
13. Olin AC, 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**:1319–25.
14. Olivieri M, Talamini G, Corradi M, et al. Reference values for exhaled nitric oxide (reveno) study. *Resprir Res* 2006;**7**:94.
15. Buchvald F, Baraldi E, Carraro S, et al. Measurements of exhaled nitric oxide in healthy subjects age 4–17 years. *J Allergy Clin Immunol* 2005;**115**:1130–6.
16. Haight RRGR, Brooks SM. The effects of age on exhaled breath nitric oxide levels. *Lung* 2006;**184**(113):119.
17. Olin AC, Bake B, Toren K. Fraction of exhaled nitric oxide at 50 mL/s. *Chest* 2007;**131**:1852–6.
18. 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**: 238–42.
19. American thoracic Society documents. ATS/ERS standardization of lung function testing: standardization of spirometry. *Am J Respir Crit Care Med* 2005;**26**:319–38.
20. Horvath I, Barnes PJ. Exhaled monoxides in asymptomatic atopic subjects. *Clin Exp Allergy* 1999;**29**:1276–80.
21. The European Community Respiratory Health Survey II Steering Committee. The European Community respiratory Health Survey II. *Eur Respir J* 2002;**20**:1071–9.
22. Heffler E, Guida G, Marsico P, et al. Exhaled nitric oxide as a diagnostic test for asthma in rhinitic patients with asthmatic symptoms. *Respir Med* 2006;**100**:1981–7.
23. Lopuhaa CE, Koopmans JG, Jansen HM, van der Zee JS. Similar levels of nitric oxide in exhaled air in non-asthmatic rhinitis and asthma after bronchial allergen challenge. *Allergy* 2003; **58**:300–5.
24. Chambers DC, Tunnicliffe WS, Ayres JG. Acute inhalation of cigarette smoke increases lower respiratory tract nitric oxide concentrations. *Thorax* 1998;**53**:677–9.
25. Malinovschi A, Janson C, Holmkvist T, Norbäck D, Meriläinen P, Högman M. Effect of smoking on exhaled nitric oxide and flow-independent nitric oxide exchange parameters. *Eur Respir J* 2006;**28**:339–45.
26. Robbins RA, Millatamal T, Lassi K, Rennard S, Daughton D. Smoking cessation is associated with an increase in exhaled nitric oxide. *Chest* 1997;**112**:313–8.
27. Hogman M, Holmkvist T, Walinder R, et al. Increased nitric oxide elimination from the airways after smoking cessation. *Clin Sci* 2002;**103**:15–9.
28. Verleden GM, Dupont LJ, Verpeut AC, Demedts MG. The effect of cigarette smoking on exhaled nitric oxide in mild steroid-naive asthmatics. *Chest* 1999;**116**:59–64.
29. Proud D. Nitric oxide and the common cold. *Curr Opin Allergy Clin Immunol* 2005;**5**:37–42.
30. de Gouw H, Grünberg K, Schot R, Kroes A, Dick E, Sterk P. Relationship between exhaled nitric oxide and airway hyperresponsiveness following experimental rhinovirus infection in asthmatic subjects. *Eur Respir J* 1998;**11**:126–32.
31. Malmberg LP, Petays T, Haahtela T, et al. Exhaled nitric oxide in healthy nonatopic school-age children: determinants and height-adjusted reference values. *Pediatr Pulmonol* 2006(41).
32. Grönke L, Kanniess F, Holz O, Jörres RA, Magnussen H. The relationship between airway hyper-responsiveness, markers of

inflammation and lung function depends on the duration of the asthmatic disease. *Clin Exp Allergy* 2002;**32**.
33. Berry MA, Shaw DE, Green RH, Brightling CE, Wardlaw, Pavord ID. The use of exhaled nitric oxide concentration to identify eosinophilic airway inflammation: an observational study in adults with asthma. *Clin Exp Allergy* 2005;**35**:1175–9.
34. Müller K, Jörres R, Magnussen H, Holz O. Comparison of exhaled nitric oxide analysers. *Respir Med* 2005;**99**:631–7.
35. 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**:1392–6.