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Effects of growth and aging on the reference values of pulmonary nitric oxide dynamics in healthy subjects

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Abstract

The lung just like all other organs is affected by age. The lung matures by the age of 20 and age related changes starts around middle age, 40-50 years. Exhaled nitric oxide (F\textsubscript{E}NO) has been shown to be age, height and gender dependent. We hypothesize that the nitric oxide (NO) parameters alveolar NO (C\textsubscript{A}NO), airway flux (J\textsubscript{aw}NO), airway diffusing capacity (D\textsubscript{aw}NO) and airway wall content (C\textsubscript{aw}NO), will also demonstrate this dependence.

Data from healthy subjects were gathered by the current authors from their earlier publications in which healthy individuals were included as control subjects.

Healthy subjects (n=433) ranged in age from 7 to 78 years. Age stratified reference values of the NO parameters were significantly different. Gender differences were only observed in the 20-49 age group. The results from the multiple regression models in subjects older than 20 years revealed that age, height and gender interaction together explained 6% of variation in F\textsubscript{E}NO at 50 mL/s (F\textsubscript{E}NO\textsubscript{50}), 4% in J\textsubscript{aw}NO, 16% in C\textsubscript{aw}NO, 8% in D\textsubscript{aw}NO and 12% in C\textsubscript{A}NO.

In conclusion, in this study we have generated reference values for NO parameters from an extended NO analysis of healthy subjects. This is important in order to be able to use these parameters in clinical practice.
Introduction

The use of non-invasive methods to diagnose respiratory diseases and monitor the treatment is advantageous for both patients and healthcare professionals. Exhaled nitric oxide (FeNO) has been used extensively since its discovery in human breath [1], especially in asthma where clinical practice guidelines have already been published [2]. The pulmonary nitric oxide dynamics models have the advantage of being a more precise assessment of nitric oxide (NO) dynamics, but their application has been limited [3]. The technical development has rapidly evolved and today we have NO analysers adopted for clinical use, both in specialized respiratory medicine and primary care [4, 5].

FeNO from one single exhalation will give a measured value the NO production from the entire respiratory system. A more detailed insight can be gained through the use of the mathematical two-compartment model (2CM) of pulmonary NO dynamics, which differentiates the NO exchange of the peripheral and central parts of the lung and explains the flow dependence of FeNO [6, 7]. In brief, the 2CM consists of an alveolar compartment comprising the peripheral gas exchanging parts of the lung (respiratory bronchioles and alveoli) and an airway compartment comprising the conductive airways larger than respiratory bronchioles. Gas in the alveolar compartment holds a certain concentration of NO (C_A NO). During exhalation, alveolar gas travels through the bronchial compartment and more NO diffuses from the bronchial wall into the luminal gas (airway NO-flux, J_2wNO) [8]. C_A NO and J_2wNO can be estimated based on a linear model if FeNO is measured at three flow rates at least of 100 mL/s [9]. If a flow rate less than 30 mL/s is used together with a median and a high flow rate, i.e. 100 and 300 mL/s, then a non-linear model can be applied which also estimates the airway wall concentration of NO (C_2wNO) and the diffusing capacity of NO from the airway wall to the gas stream (D_2wNO) [8, 10]. Investigations have used the 2CM with interesting results especially for C_A NO where increased values have been found in severe asthma [11], alveolitis [12], and chronic obstructive pulmonary disease [10, 13] and early scleroderma [14]. C_A NO has been specifically used to identify scleroderma patients at high risk for lung function deterioration and advancing disease, with 5.3 ppb being suggested as the cut off value [15].
References values are necessary for any new method to be accepted in clinical practice and reference values for $F_{E\text{NO}}$ at the recommended flow of 50 mL/s ($F_{E\text{NO}_{50}}$) are published [16, 17]. Height, age and gender have been shown to influence $F_{E\text{NO}_{50}}$. Reference values for NO parameters from extended NO analysis are limited to two publications, one with 89 adults [18] and one with 66 children [19]. The lung matures by the age of 20 in regard to closing volume [20] and in older age the diffusing capacity declines in a linear fashion with increasing age [21], and these changes in pulmonary physiology might also affect NO parameters. The aim of this study was to establish reference values for NO parameters in healthy subjects ranging from young to old age.

**Methods**

**Subjects**

Data from healthy non-smoking subjects were gathered by the current authors from their earlier publications in which healthy individuals were included as control subjects [10, 14, 18, 19, 22-30]. In the majority of these studies measurements of lung function and symptom questionnaires verified the health status. Gender, age and height were noted. Exhaled flow together with corresponding exhaled NO levels were collected.

**NO analysis**

$F_{E\text{NO}_{50}}$ and $F_{E\text{NO}}$ values from exhalation with flows 5 – 500 mL/s for the extended NO analysis were gathered. All data were recalculated either with the linear model (Tsoukias & George, TG) [9] using three flow rates at least 100 mL/s or with the non-linear model (Högman-Meriläinen Algorithm, HMA) [10, 22] using a low flow rate of 5, 10 or 20, a median rate of 100 and a high flow rate of 300, 400 or 500 mL/s. Data were fed into an algorithm in a standard Microsoft® Excel environment for the estimation of the NO parameters (*on-line material*). When generating NO parameters from the linear model [9] Pearson’s r-value was noted. With the use of NO parameters from the non-linear model [10, 22] a plot of flow with corresponding NO values can be generated and at the flow of 50 mL/s, a NO value was noted and compared to the measured $F_{E\text{NO}_{50}}$ for a quality control of the estimation of the NO parameters. With the non-linear model there is also a built in quality test of the curve [10]. This is in line with the first guidelines for the extended NO analysis [31].
**Statistical analysis**

Due to aging of the lung, the subjects were divided into three age groups <20 years, 20-49 years and ≥50 years. Descriptive data of the subjects are presented as frequency or as medians and quartiles where appropriate. The distributions of the NO parameters, stratified by age groups, are presented as arithmetic mean or geometrical mean (for skewed distributed data) and as 2.5th, 5th, 25th, 50th, 75th, 95th, 97.5th percentiles. Kruskal-Wallis test and one-way ANOVA were used to test for differences in the distribution of NO parameters between the age groups. In the case of significant difference between age groups, post-hoc tests were performed using a pairwise Mann-Whitney U-test. Pearson Correlation was used to test correlations to $C_A\text{NO}$. Spearman's rank order correlation was use for the other NO-parameters.

Gender stratified simple regression models were fitted with the logarithms of $F_{E\text{NO}}^{50}$, $C_{aw\text{NO}}$, $D_{aw\text{NO}}$, and $J_{aw\text{NO}}$, respectively, as dependent variable, and with age as independent variable. Logarithmically scaled regression lines were retransformed back into natural scale and all regression lines were then plotted along with their corresponding 95 % reference intervals.

Multiple regression modelling was performed on data where subjects younger than 20 years were excluded, as the children differ from adults in the relationship between height and age that made if difficult to fit robust statistical models. The models were specified with the $C_A\text{NO}$ in natural scale, the logarithms of $F_{E\text{NO}}^{50}$, $C_{aw\text{NO}}$, $D_{aw\text{NO}}$, and $J_{aw\text{NO}}$, respectively as dependent variable, and with age, height and gender, including interaction terms between gender*height and gender*age as independent variables. For all the models, ANOVA chunk tests were performed, jointly testing if the two interaction terms contributed significantly to the models as compared to omitting them from the model. As this was not the case for any of the NO-parameters, the models were refitted without the interaction terms. To account for potential cluster effect in the data, we also controlled for study centre and estimation method (TG vs. HMA). To help interpretability of regression coefficients, the variables age and height were centred and age was scaled to a unit of 10 years and 10 cm respectively [32]. For the factor gender, B represents the expected ratio in geometrical means between a male and a female, keeping all other variables fixed. For the logarithmically transformed parameters, regression coefficients have been retransformed to natural scale using the exponential function. The bootstrap procedure produces optimism-corrected estimates of $R^2$, with a
correction factor based on the average difference, in over 5000 bootstrap samples, between the \(R^2\) of the model fit to the bootstrap data and the \(R^2\) of the bootstrap model applied to the original data.

Model assumptions of normality and homoscedasticity of residuals were assessed from graphs. A p-value < 0.05 was considered statistically significant. Excel (Microsoft\textsuperscript{R} Office 2011) was used for calculations of the NO parameters. Statistical analyses were performed using SPSS, v. 22 (SPSS Inc., Chicago, MI, USA), and R [33] using the rms package [34].

**Results**

Healthy subjects (n=433) aged between 7 – 78 years were analysed. There were more men (n=268) than women (n=165) (Table 1). There was no difference in \(F_{E\text{NO}}\) between the study centres (p=0.37).

Table 1. Subject characteristics in the different age groups presented by gender.

| Age group | <20 yrs | 20-49 yrs | ≥50 yrs |
|-----------|---------|-----------|---------|
| Gender    | Female  | Male      | Female  | Male      | Female  | Male      |
| Subjects, n | 41      | 42        | 82      | 113       | 42      | 113       |
| Age, years| 10 (9,11) | 10 (8,12) | 33 (26,40) | 39 (30,44) | 53 (52,60) | 56 (52,65) |
| Height, m | 1.39 (1.32,1.47) | 1.37 (1.31,1.49) | 1.68 (1.64,1.71) | 1.81 (1.75,1.85) | 1.67 (1.63,1.69) | 1.76 (1.72,1.80) |
| Weight, kg | 34 (30,38) | 32 (28,39) | 60 (55,68) | 80 (73,87) | 70 (61,76) | 79 (73,88) |
| BMI, kg/m\(^2\) | 17 (16,19) | 17 (16,19) | 22 (20,23) | 25 (23,26) | 25 (23,27) | 26 (24,28) |

*Data are given in median (25,75 percentile)*

The NO parameters were estimated using the linear model TG (n=87) with an r-value from 0.90 to 1.0, and with a median value of 1.0 (0.99, 1.0). In the non-linear model HMA (n=346), all passed the built in quality test. The difference in measured and estimated \(F_{E\text{NO}}\) ranged from -5.0 to 5.0 with a median value of 0.3 (-0.6, 1.3) ppb.
NO parameters in the different age groups

There were statistically significant differences in the distribution of the NO parameters between the young, middle and older age groups (Table 2). FE\textsubscript{NO\textsubscript{50}} was higher in the older age group compared to the young age group (p<0.001) and the middle age group (p=0.001), and FE\textsubscript{NO\textsubscript{50}} was higher in the middle age group than the younger age group (p<0.001). J\textsubscript{aw}NO was lower in the young age group compared to the middle age (p<0.001) as well as the older age group (p<0.001). C\textsubscript{aw}NO was higher in the older age group compared to the young age group (p<0.001) and the middle age group (p<0.001), and C\textsubscript{aw}NO was higher in the middle age group than in the younger age group (p<0.001). D\textsubscript{aw}NO was lower in the older age group compared to the young age group (p=0.023) and the middle age group (p=0.001). C\textsubscript{A}NO was lower in the middle age group compared to the young age group (p=0.001) and the older age group (p<0.001).
Table 2. Mean values and percentile distribution of FE\textsubscript{NO}_{50} and NO parameters in the three age groups.

| Age groups | Mean | p-value* | 2.5 | 5 | 25 | 50 | 75 | 95 | 97.5 |
|------------|------|---------|-----|--|----|----|----|----|-----|
| FE\textsubscript{NO}_{50}, ppb |      |         |     |  |    |    |    |    |     |
| <20 yrs    | 10.8 | <0.001  | 4.7 | 4.9 | 7.1 | 10.5 | 15.9 | 25.6 | 27.0 |
| 20-49 yrs  | 16.0 |         | 6.6 | 7.4 | 12.0 | 15.3 | 20.9 | 38.0 | 45.5 |
| ≥50 yrs    | 18.2 |         | 7.7 | 8.5 | 13.2 | 18.2 | 25.3 | 36.5 | 44.9 |
| J\textsubscript{av}NO, nL/s |      |         |     |  |    |    |    |    |     |
| <20 yrs    | 0.40 | <0.001  | 0.08 | 0.10 | 0.26 | 0.38 | 0.66 | 1.36 | 1.60 |
| 20-49 yrs  | 0.76 |         | 0.31 | 0.34 | 0.53 | 0.70 | 1.08 | 2.01 | 2.51 |
| ≥50 yrs    | 0.81 |         | 0.27 | 0.31 | 0.52 | 0.83 | 1.23 | 2.00 | 2.23 |
| C\textsubscript{av}NO, ppb |      |         |     |  |    |    |    |    |     |
| <20 yrs    | 64   | <0.001  | 19  | 21 | 34 | 58 | 123 | 208 | 439 |
| 20-49 yrs  | 105  |         | 30  | 35 | 65 | 105 | 160 | 309 | 441 |
| ≥50 yrs    | 155  |         | 40  | 51 | 89 | 150 | 267 | 491 | 535 |
| D\textsubscript{av}NO, mL/s |      |         |     |  |    |    |    |    |     |
| <20 yrs    | 7.5  | 0.002   | 0.9 | 1.3 | 4.8 | 8.7 | 13.1 | 25.6 | 26.9 |
| 20-49 yrs  | 7.8  |         | 1.3 | 2.5 | 5.3 | 8.3 | 12.7 | 19.1 | 21.6 |
| ≥50 yrs    | 5.7  |         | 1.0 | 1.2 | 3.5 | 6.2 | 10.3 | 17.1 | 20.8 |
| C\textsubscript{NO}, ppb |      |         |     |  |    |    |    |    |     |
| <20 yrs    | 2.07 | <0.001  | 0.11 | 0.61 | 1.52 | 2.05 | 2.73 | 3.59 | 3.88 |
| 20-49 yrs  | 1.72 |         | 0.21 | 0.29 | 1.13 | 1.61 | 2.23 | 3.66 | 3.93 |
| ≥50 yrs    | 2.2  |         | 0.33 | 0.51 | 1.48 | 2.25 | 2.85 | 3.77 | 3.88 |

Data with skewed distribution are given in geometrical mean\textsuperscript{1}, *Kruskal-Wallis test for difference in mean values between age groups.
NO parameters in the different age groups by gender

There was only a difference between genders in the middle age group in \( \text{FeNO}_{50} \) (\( p<0.001 \)), \( J_{awNO} \) (\( p<0.001 \)), \( C_{awNO} \) (\( p<0.001 \)) and \( C_{A}NO \) (\( p=0.027 \)) but not in \( D_{awNO} \) (Table 3).

Table 3. \( \text{FeNO}_{50} \) and NO parameters in the different age groups presented by gender.

| Age group | <20 yrs | 20-49 yrs | ≥50 yrs |
|-----------|---------|-----------|---------|
| Gender    | Female  | Male      | Female  | Male    | Female  | Male    |
| \( \text{FeNO}_{50}, \) ppb | 11 (8,16) | 10 (7,15) | 13 (10,17) | 18 (13,23)* | 17 (12,23) | 19 (14,26) |
| \( J_{awNO}, \) nL/s | 0.43 (0.28,0.66) | 0.37 (0.23,0.66) | 0.63 (0.44,0.83) | 0.87 (0.60,1.25)* | 0.75 (0.49,1.14) | 0.84 (0.54,1.26) |
| \( C_{awNO}, \) ppb | 76 (48,130) | 54 (30,84) | 77 (54,115) | 126 (77,211)* | 121 (71,173) | 163 (100,288) |
| \( D_{awNO}, \) mL/s | 6.5 (4.0,10.9) | 8.7 (5.5,17.4) | 8.8 (6.2,12.8) | 7.3 (4.8,12.7) | 7.1 (4.8,11.4) | 5.4 (3.2,9.8) |
| \( C_{A}NO, \) ppb | 2.12 (1.78,2.39) | 1.98 (1.25,2.33) | 1.99 (1.22,2.39) | 1.52 (1.07,2.06)* | 2.44 (1.25,2.92) | 2.20 (1.45,2.83) |

Data are given in median (25,75 percentile). \(^1\)Geometrical mean. Mann-Whitney U-test for gender differences, *\( p<0.05 \).

Regression analyses

Relationships between age and the NO-parameters (\( J_{awNO}, C_{A}NO, D_{awNO} \) and \( C_{awNO} \)), with univariate regression lines and estimated 95 % reference intervals are shown in Figure 1.
The multiple regression analyses, with the bootstrap validation step, showed...

- **CawNO (ppb)**: 
  - Age vs. CawNO (ppb) graph with a trend line.

- **JawNO (mL/s)**: 
  - Age vs. JawNO (mL/s) graph with a trend line.

- **JawNO (nL/s)**: 
  - Age vs. JawNO (nL/s) graph with a trend line.

- **DawNO (mL/s)**: 
  - Age vs. DawNO (mL/s) graph with a trend line.
Figure 1. Relationship between age and the NO-parameters, airway NO flux (J\textsubscript{aw}NO), alveolar NO (C\textsubscript{A}NO), airway diffusing capacity (D\textsubscript{aw}NO) and airway wall content (C\textsubscript{aw}NO), with univariate regression lines and estimated 95% reference intervals. Since children differ markedly from adults, in particular regarding the associations between height and age, the young age group was treated separately.

The multiple regression analyses, with the bootstrap validation step, showed in the age groups above 20 years that age, height and gender interactions together explained 6% of variation in F\textsubscript{E}NO\textsubscript{50}, 4% in J\textsubscript{aw}NO, 16% in C\textsubscript{aw}NO, 8% in D\textsubscript{aw}NO and 12% in C\textsubscript{A}NO (Table 4). Age was a significant predictor in all models p<0.001 except for J\textsubscript{aw}NO (p=0.18) (Table 4). The association was positive for F\textsubscript{E}NO\textsubscript{50} and all NO parameters. Gender contributed as significant main effects for C\textsubscript{aw}NO and C\textsubscript{A}NO only. Multiple linear regression models poorly predicted the large variations in F\textsubscript{E}NO\textsubscript{50} and NO parameters.

In the age group <20 years there were only 83 subjects and therefore multiple regression models were not applied. Age correlated positively to F\textsubscript{E}NO\textsubscript{50} (r=0.31, p=0.005) and to J\textsubscript{aw}NO (r=0.32, p=0.003). There were stronger correlations between height and F\textsubscript{E}NO\textsubscript{50}, r=0.45, p<0.001, and height and J\textsubscript{aw}NO r=0.41, p=0.001, while no correlations were found between height and C\textsubscript{A}NO, C\textsubscript{aw}NO and D\textsubscript{aw}NO.

Table 4. Regression coefficients (B) and p-values of the multiple regression models for NO-variables. The R\textsuperscript{2} is the unadjusted coefficient of determination of the models and R\textsuperscript{2}\textsubscript{boot} is the corresponding optimism-corrected R\textsuperscript{2} values as estimated by bootstrapping.

|                | Intercept | Age | Height | Gender (male) | R\textsuperscript{2} | R\textsuperscript{2}\textsubscript{boot} |
|----------------|-----------|-----|--------|---------------|----------------------|-----------------------------------------------|
| F\textsubscript{E}NO\textsubscript{50} ppb | 15.8      | 1.07| <0.001 | 1.04          | 0.29                 | 1.12 0.13 0.08 0.06                           |
| J\textsubscript{aw}NO nL/s | 0.77      | 1.03| 0.18   | 1.05          | 0.33                 | 1.10 0.26 0.07 0.04                           |
| C\textsubscript{aw}NO ppb | 86.6      | 1.16| <0.001 | 0.87          | 0.04                 | 1.70 <0.001 0.19 0.16                         |
| D\textsubscript{aw}NO mL/s | 8.6       | 0.88| <0.001 | 1.21          | 0.01                 | 0.69 0.01 0.11 0.08                           |
| C\textsubscript{A}NO ppb | 1.95      | 0.2 | <0.001 | -0.03         | 0.68                 | -0.24 0.09 0.15 0.12                          |
Discussion

In this study we have generated reference values for NO parameters from an extended NO analysis of healthy subjects. By pooling the healthy subjects' data from earlier published data the values of NO parameters for a large group of subjects can be presented. We have found that age influences $F_{E}NO$ and all the NO parameters, while gender affects NO parameters only in the middle age group. Multiple linear regression models poorly predicted the large variations in $F_{E}NO_{50}$ and NO parameters.

In the See et al. paper (n=13.275) about 10% of the variation in $F_{E}NO$ was explained by a variety of variables [35], and this is in line with the current results (n=433) where about 6% of the variation in $F_{E}NO_{50}$ was explained by age, height, gender, NO model and study centre.

Lung development

In the under 20-age group, $F_{E}NO_{50}$ and the airway NO parameters $J_{aw}NO$ and $C_{aw}NO$ were lower than in the other age groups. This could possibly reflect an increasing mucosal surface area with increasing height and growing lung volumes. This was also present in the study by Jacinto et al. where the $F_{E}NO_{50}$ increase breakpoint appeared around 14 years in girls and 16 years in boys [17]. This is in line with the growth of the body, and more specifically the development of the bronchial tree.

Ageing

In the middle and older age groups pulmonary aging seems to increase $C_{A}NO$. This possibly reflects decreased diffusivity of gases in the distal portion of the lung, as $C_{A}NO$ is determined not only by factors producing NO in the lung periphery but also by how much alveolar NO can diffuse into the pulmonary circulation where it is rapidly scavenged by haemoglobin. In older age, the diffusing capacity declines in a linear fashion with increasing age [21] and in elderly healthy subjects there is a decrease in steady-state transfer capacity for carbon monoxide (CO) [36] and NO [37]. There is also an increase in residual volume [38] reflecting obstruction of the distal part of the airways that could possibly contribute to the increase in $C_{A}NO$ seen in this study. Thus, there is an accumulation of NO from the alveolar region together with the inhaled NO from the airways, which increases with age, and both can contribute to the increase of
C₄NO. However, the uptake of NO in pulmonary capillaries is very high [39], and the increase in C₄NO could also be due to other causes. One of these other causes affecting C₄NO may be that clinically healthy older subjects have an altered inflammatory cell profile and can actually have a low-grade inflammation in the lower respiratory tract [40]. This could be due to the macrophages becoming less efficient in scavenging invading microorganisms in older age groups [41, 42]. This can be an explanation for the increased exhaled FₑNO₅₀ and NO parameters, i.e. JₑwNO, CₑwNO and C₄NO in our older subjects.

In studies with older unhealthy patients, it is important that the control subjects be matched to them by age until there is enough data for this age group. Therefore, the increased C₄NO that has been found in COPD patients should be re-evaluated since they have been compared in some studies to younger individuals [10, 13]. However, in other studies with, i.e. in systemic sclerosis or alveolitis the C₄NO values are surely increased since there were no age differences between the patients and control subjects [12, 14, 43]. Matching by gender should also be taken into account for the middle age group, since C₄NO increases earlier in females. This is possibly explained by a decrease in capillary blood volume of the lung [44] causing an impaired gas exchange in women in the middle age group.

DₑwNO decreased with increasing age. This is interesting, as DₑwNO is the total diffusivity of NO from bronchial mucosa to luminal air, and it can be assumed to reflect both the total surface area available for diffusion and also the physical properties of the mucosa affecting the diffusivity of gases. As individuals grow so do their bronchial trees, and one would assume that DₑwNO increases with increasing height, but we did not see this. Instead, we found that CₑwNO increased and this explained the increase in JₑwNO and FₑNO₅₀ during the growth period. The decrease of DₑwNO found in older age might reflect the physical changes occurring in the bronchial mucosa of the aging lung.

**Gender**

It was only in the middle age group where a gender difference could be found in FₑNO₅₀, JₑwNO, CₑwNO and C₄NO. In the regression model only the variations in CₑwNO and C₄NO were significant for gender.

Olin *et al.* found FₑNO₅₀ to be higher in men than in women around 50 years of age with 18 resp. 15 ppb respectively, but when comparing FₑNO₅₀ between the sexes with
similar heights and ages no difference was found [16]. Jacinto et al. have shown a gender difference in the same age group with men slightly above 15 ppb and women around 12 ppb [17]. The corresponding values for $FE\text{NO}_{50}$ in the present study with the young age group excluded are 16 ppb for men and 15 ppb for women, which are in line with the values obtained by Olin et al. using the same analysing method, namely chemiluminescence.

A limitation in this study is that data were pooled, which resulted in more men than women, especially in the old age group. In addition, the cross-sectional design of the study is not optimal to assess relation between age and NO parameters. However, long enough longitudinal studies would require decades of follow-up. It would be interesting to put lung function in relation to the NO parameters, but unfortunately we did not have lung function data from all of the subjects. We did check that there was no significant difference in the mean $FE\text{NO}_{50}$ values between the different centres, which suggests that the methodology was similar enough to allow for the pooling of the data.

In conclusion, in this study we have generated reference values for NO parameters from an extended NO analysis of healthy subjects. This is important in order to be able to use these parameters in clinical practice. We found that pulmonary aging seems to increase $CA\text{NO}$, which is possibly a reflection of a decreased diffusivity of gases in the gas exchange area. The impaired immune defence system that occurs with old age could also explain the increase in all NO parameters except $D_{ln}\text{NO}$ that was decreased in this group. Further studies or additional pooling of data are needed before we can provide even better age related reference values for the NO parameters and possibly create reliable reference equations. However, this is currently the largest dataset for NO parameters that can be used as a basis for comparisons in future studies regarding health and disease.
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