Lung (2019) 197:73–80
https://doi.org/10.1007/s00408-018-0188-5

ASTHMA

The Effects of Aging on Exhaled Nitric Oxide (FeNO) in a North African Population

Sonia Rouatbi[1,2,3] · Ines Ghannouchi[1,2] · Helmi Bensaad[1,2,3]

Received: 31 July 2018 / Accepted: 16 December 2018 / Published online: 14 January 2019

Abstract

Objective To determine and explain the effect of age on exhaled nitric oxide values in North African healthy subjects aged from 5 to 83 years.

Design Prospective cross-sectional study.

Methods Volunteer children adults and elderly healthy subjects were included. A medical questionnaire was used to assess several subject characteristics. The levels of exhaled fraction of nitric oxide (FeNO) were measured by Medisoft HypAir FeNO method. Spirometry function test was done after the FeNO measurement. The following parameters were measured: forced vital capacity (FVC, L); 1st second forced expiratory volume (FEV_1, L); FEV_1/FVC ratio (absolute value); maximal mid expiratory flow (MMEF, L/s); Mid expiratory flow from 25 to 75% (MEF25%, MEF50%, and MEF75%). Statistical analyses were carried out using Statistica software with a significance set at the 0.05 level.

Results A significant increase in FeNO is noted between groups with respective age ranges of (5, 17) and (17, 25) years with a breakpoint at 1,397,034 years. A significant decrease of FeNO is noted between groups with respective age ranges of (45, 55) and (55, 65) years with a breakpoint at 6,366,052 years. No statistical significant difference was found between females' and males' means FeNO data. Finally, SEL, obesity status, and hypertension contribute significantly in the variations of FeNO values.

Conclusion The development and aging of the lung touched non-respiratory functions and so modified FeNO values in healthy North African subjects.

Keywords Lung aging · Exhaled nitric oxide · North Africa · Lung development

Introduction

The measurement of fractional exhaled nitric oxide concentration (FeNO) has been proposed as a useful biomarker for monitoring and management of inflammatory airway diseases [1]. Asthma is one of the most common and serious respiratory diseases and is characterized by chronic airway inflammation [2]. During the process of airway inflammation, nitric oxide (NO) synthesis is increased by eosinophilic infiltration. So FeNO can be considered as an "inflammometer" of the bronchi.

General population studies have reported that FeNO levels are affected by various demographic and clinical factors such as age [3, 4], gender [5, 6] height [3, 4, 6], atopy [3, 6, 7], and smoking status [6, 7]. The levels of FeNO were also be affected by racial differences [8].

Recently, the FeNO values of a large group of healthy Tunisian/Arab children, adults, and elderly subjects were prospectively measured. These studies showed that FeNO values are age dependent [9–11]. These changes in FeNO values could be explained in part by the development and aging of the lung. Development of the lung can be divided into two phases, lung growth (structural development) and lung maturation (functional development). Lung growth can be influenced by a host of physical factors. Lung maturation and the achievement of functionality is primarily a

✉ Sonia Rouatbi
sonia.rouatbi@gmail.com

1 Department of Physiology and Functional Explorations, Farhat HACHED Hospital, Sousse, Tunisia

2 Laboratory of Physiology, Faculty of Medicine of Sousse, University of Sousse, Street Mohamed Karoui, 4000 Sousse, Tunisia

3 Heart Failure (LR12SP09) Research Laboratory, Farhat HACHED Hospital, Sousse, Tunisia

biochemical process and is under the control of a number of different hormones [12, 13]. As the lung gets into a completely new environment at birth, transformations are necessarily performed in a functioning organ; it can be expected that the environment may have some regulatory influence on the processes governing lung growth. An effect of elevated partial pressure of oxygen, appeared to be the reason for the observed reduction in alveolar surface area [14]. Lung development is regulated by many mediators and hormones. Nitric oxide (NO) is one of these mediators. It is synthesized from l-arginine via three NO synthase (NOS) isoenzymes: endothelial NOS (eNOS) and neuronal NOS are expressed in endothelial cells and neurons, respectively, and generate small amounts of NO on activation by Ca^{2+}, whereas inducible NOS (iNOS) is induced by various proinflammatory cytokines. NO mediates proliferation, migration, and differentiation of endothelial cells [15, 16] and interacts with multiple angiogenic growth factors [17].

Both eNOS- and iNOS-knockout mice show severely impaired postpneumonectomy lung growth; treatment of wild-type mice with the NOS inhibitor N^G-nitro l-arginine methyl ester has the same effect suggesting specific growth impairment caused by NO deficiency [17].

Lung development finished with adulthood giving way to lung aging with unspecified ages of the end of lung development and of the beginning of aging, respectively [12]. Several studies have shown that lung aging can touch respiratory and non-respiratory functions and pulmonary function measurements were a good tool for exploring lung aging [18, 19]. NO production by pulmonary tract, which is a non-respiratory function, could be influenced by age and so FeNO could constitute a good tool for studying lung aging.

Therefore, the present study aims (1) to identify clinical (physical activity, socioeconomic and instruction levels, diabetes, and High blood pressure), anthropometric (age, height, weight, and obesity status), and functional (spirometric parameters) factors that influence the development and aging of the lung "Inflammometer" (FeNO values), (2) to determine the cut-off points of FeNO values corresponding to the end of lung development and the beginning of aging, respectively.

Methods

Study Design

It is a cross-sectional study spread over 12 months. It was conducted at the Farhat HACHED Hospital, Sousse, Tunisia. Study design consists of a convenience sample of healthy Tunisian subjects aged 05–83 years (Arab race).

Study approval was obtained from the hospital ethics committee and written informed consent was obtained from all subjects.

Subjects

Volunteer children (parent's consent) adults and elderly healthy subjects were included. The following non-inclusion criteria were applied: to have fever or chronic illnesses especially cardiovascular diseases; otorhinolaryngologic diseases or symptoms (allergic rhinitis, symptoms, and signs of acute respiratory infection during 2 weeks prior to assessment); clinical manifestation of allergic diseases (urticaria, skin allergy, atopic dermatitis, or eczema); a history of pulmonary diseases or related respiratory symptoms (history of asthma, current or past symptoms of wheeze or chronic cough, and chronic obstructive pulmonary disease); abnormal lung function data; regular medication (glucocorticoid, bronchodilator…); current or ex-smokers (cigarettes or narghile use and inability to perform properly FeNO or spirometry measurements [11, 20]).

Medical Questionnaire

A medical questionnaire [20] was used to assess several subject characteristics. Age (years) was taken as the number of complete years from birth to the date of the study. Height (cm) and weight (kg) were measured. Body mass index (BMI) was calculated ($=\text{weight}/\text{height}^2$). Two groups of subjects were defined non-obese ($\text{BMI} < 30$) and obese ($\text{BMI} \geq 30$) [21].

FeNO Measurement

The FeNO (parts per billion, ppb) was measured by Medisoft HypAir FeNO method using an electrochemical analyzer (Medisoft, Sorinnes (Dinant), Belgium). The instrument was calibrated and used according to the manufacturer's instructions and work in conjunction with a personal computer. The software supplied by either manufacturer provided visual feedback allowing the participant to maintain a constant exhaled breath flow rate. Measurements were made between 8 a.m. and 12 a.m.

The online method with constant flow rate was used [22]. A nose clip was not used.

Subjects were asked not to eat, not to drink water or alcohol, and not to ingest caffeine nor participate in strenuous activities for 2 h prior to the test [22].

Three acceptable measurements (within 10%) were taken at the recommended flow rate of 50 mL/s within a 15-min period [22].

Spirometry Function Test

According to the international recommendations [23], spirometry was carried out in the sitting position, and a nose clip was applied. All tests were performed by the same investigator using a spirometer (ZAN 100, Messgerate GmbH, Oberthulba, Germany). They were done after the FeNO measurement [22]. The flow sensor of the spirometer was calibrated daily with a 3-L syringe.

The following parameters were measured: forced vital capacity (FVC, L); 1st second forced expiratory volume (FEV_1, L); FEV_1/FVC ratio (absolute value); maximal mid expiratory flow (MMEF, L/s); Mid expiratory flow from 25 to 75% (MEF25%, MEF50%, and MEF75%).

The results were compared with local age- and sex-matched reference values [24]. Obstructive ventilatory defects were retained when the FEV_1/FVC ratio was lower than the lower limit of normal (LLN). FEV_1 and FVC were considered as abnormal when they were lower than the LLN [23].

Statistical Analysis

For each subject, the mean of the three correct FeNO values was used for statistical analysis.

Preliminary descriptive analysis included frequencies for categorical variables (sex: male/female) and obesity status (non-obese/obese) and means ± standard deviation (SD) and 95% confidence interval (95% CI) for continuous ones (anthropometric, spirometric, and exhaled nitric oxide data).

Since the distribution of the dependent variable (FeNO) was normally distributed, FeNO results were presented as mean ± SD (95% CI), and as minimum–maximum.

All analyses were performed without outliers, defined as FeNO values above arithmetic mean ± 3SD [22].

Univariate Regression Analysis: Influencing Factors

t Tests were used to evaluate the associations between FeNO and the categorical variables (gender, age, age ranges, physical activity, obesity status, instruction levels, socioeconomic levels, diabetes, and hypertension).

Comparison of FeNO values with student *t* test for independent samples by groups. Grouping variables are physical activity: PhA (sedentarity = 0, active = 1), obesity status: obesity (Obese = 1, non-obese = 0), instruction levels: IL (hight = 1, Low = 0), socioeconomic levels: SEL (hight = 1, low = 0), diabetes (1, 0), and hypertension (hypertension = 1, non-hypertension = 0).

Piecewise Linear Regression with Breakpoint

Piecewise linear regressions analyses were used to determine the breakpoint (the point from which the FeNO increase or drop depending on age begins). The estimation method used is Quasi-Newton.

Analyses were carried out using Statistica (Statistica Kernel version 6, StatSoft, Maisons-Alfort, France). Significance was set at the 0.05 level.

Results

Subject's Data

Five hundred and ninety (275 males) subjects were included in the present study.

The anthropometric, spirometric, FeNO data, and obesity status of the 590 subjects are shown in Table 1. No statistical significant difference was found between females' and males' means FeNO data.

Clinical factors were tested to evaluate their contribution in the variations of the FeNO values. The comparisons of FeNO values according to physical activity (PhA: sedentarity = 0, active = 1), Instruction levels (IL: hight = 1, low = 0), and diabetes (1,0) showed that FeNO levels were unaffected by these settings ($P > 0.05$). However, socioeconomic levels (SEL: hight = 1, low = 0), high blood pressure (hypertension = 1, non-hypertension = 0), and obesity status (obesity: obese = 1, non-obese = 0) contributed significantly in FeNO modifications ($p < 0.05$). Obese patients had a significant higher value of FeNO than non-obese patients with a significant correlation between FeNO values and BMI ($r = 0.12$; $P < 0.05$).

By studying the effect of anthropometric and spirometric variables on FeNO values, a significant correlation ($P < 0.05$) was found between FeNO and the following anthropometric and spirometric parameters: age ($r = 0.14$), FEV1/FVC ratio ($r = -0.22$), MEF50 (l/s) ($r = -0.10$), and MEF50% ($r = -0.01$), MEF25 (l/s) ($r = -0.09$), MMEF (l/s) ($r = -0.10$), and MMEF% ($r = -0.16$).

Table 2 shows distribution by age ranges of anthropometric, spirometric, and FeNO parameters. A significant increase in FeNO values was noted between groups with respective age ranges of (5, 17) and (17, 25) years with a breakpoint at 13.97 years ($R = 0.77$, Variance explained = 60.80%).

In addition, a significant decrease of FeNO values was noted between groups with respective age ranges of (45, 55) and (55, 65) years with a breakpoint at 63.66 years ($R = 0.84$, Variance explained = 70.84%).

The distribution of the sample subjects' FeNO data according to age is presented in Fig. 1. A significant FeNO difference was found between subjects' range ages.

Table 1 Distribution by gender of anthropometric, spirometric, and FeNO parameters

Parameters	Total sample $N=590$		Female $N=315$		Male $N=275$		P value
	Mean	SD	Mean	SD	Mean	SD	
Age (years)	36.68	20.46	37.18	20.12	36.10	20.86	0.52
Height (cm)	158.42	16.16	154.54	12.45	162.87*	18.62	<0.01
Weight (kg)	64.67	21.154	63.028	19.14	66.56*	23.13	0.04
BMI (kg/m^2)	25.12	6.18	25.96	6.62	24.16*	5.49	<0.01
FVC (l)	3.12	1.03	2.75	0.74	3.55*	1.14	<0.01
FVC (%)	96.38	13.47	98.25	14.20	94.24*	12.25	<0.01
FEV1 (l)	2.60	0.81	2.33	0.63	2.91*	0.89	<0.01
FEV1 (%)	95.43	13.24	96.66	13.65	94.03*	12.64	0.01
FEV1/FVC (%)	25.21	40.50	22.88	39.63	27.88	41.38	0.13
MEF50% (l/s)	3.41	1.15	3.21	1.03	3.64*	1.24	<0.01
MEF50% (%)	83.60	21.24	82.75	21.33	84.68*	21.13	0.34
MEF25% (l/s)	1.36	0.72	1.31	0.70	1.42	0.74	0.06
MEF25% (%)	73.62	34.28	72.81	31.99	74.63	37.03	0.58
MMEF (l/s)	2.84	1.01	2.67	0.96	3.03*	1.03	<0.01
MMEF (%)	90.43	24.37	93.30	25.50	87.12*	22.60	<0.01
FeNO (ppb)	11.37	6.21	11.50	6.06	11.22	6.39	0.58

BMI body mass index, *FVC* forced vital capacity, FEV_1 1st second forced expiratory volume, FEV_1/FVC ratio (absolute value in %), *MMEF* maximal mid expiratory flow, *MEFx%* maximal expiratory flow at x% of FVC ($x=25$ or 50 or 75%), *FeNO* fraction of exhaled nitric oxide

*P value < 0.05: comparisons between males and females using t test

Table 2 Distribution by age ranges of anthropometric, spirometric, and FeNO parameters

Age ranges (years)	(5–17) $N=158$		(17–25) $N=45$		(25–35) $N=64$		(35–45) $N=86$		(45–55) $N=110$		(55–65) $N=76$		>65 $N=51$	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age (years)	10.66	3.384	21.696	2.31	29.54	3.01	40.01	2.72	50.59	3.02	58.41	2.59	71.48	5.62
Height (cm)	142.41	19.29	168.80	9.36	165.45	8.30	164.68	8.78	164.43	9.51	163.10	9.99	159.54	10.78
Weight (kg)	39.67	15.09	65.35	15.68	67.68	14.92	76.93	15.45	80.24	12.08	73.34	12.81	70.56	12.64
BMI (kg/m^2)	18.87	3.59	22.79	4.37	24.69	4.86	28.35	5.40	29.77	4.56	27.66	4.76	27.82	4.80
FVC (l)	2.31	0.92	3.95	0.80	3.77	0.80	3.61	0.76	3.45	0.86	3.14	0.83	2.56	0.75
FVC (%)	93.93	12.41	91.39	9.59	94.26	10.33	97.78	10.13	99.06	14.00	99.23	16.50	98.68	18.53
FEV1 (l)	2.09	0.78	3.41	0.69	3.24	0.67	2.90	0.54	2.74	0.67	2.47	0.61	2.01	0.60
FEV1 (%)	97.38	12.13	91.24	10.01	94.61	10.97	93.28	10.16	94.90	13.34	95.93	15.37	98.20	20.02
FEV1/ FVC (%)	91.93	6.10	0.86	0.05	0.86	0.06	0.80	0.04	0.79	0.04	0.79	0.06	0.78	0.06
MEF50% (l/s)	2.94	1.05	4.46	1.06	4.34	1.10	3.61	0.82	3.48	1.12	3.16	0.87	2.638	0.99
MEF50% (%)	84.22	17.22	88.79	16.29	91.94	20.41	81.80	17.39	82.49	22.13	81.76	22.84	76.02	25.24
MEF25% (l/s)	1.52	0.62	2.21	0.80	1.98	0.72	1.34	0.68	1.048	0.430	0.89	0.37	0.78	0.42
MEF25% (%)	92.22	19.96	89.64	28.84	90.46	31.53	70.65	36.96	62.82	23.56	62.75	27.12	78.92	50.04
MMEF (l/s)	2.66	0.96	3.97	0.98	3.79	0.95	2.98	0.68	2.69	0.84	2.39	0.69	1.91	0.71
MMEF (%)	103.38	22.91	89.24	17.40	92.88	20.33	82.40	19.77	82.00	21.64	85.71	26.07	86.91	31.85
FeNO (ppb)	5.18	2.99	12.97*	4.72	12.57	4.14	13.93	5.32	14.66	5.46	12.77**	5.34	14.27	7.56

BMI body mass index, *FVC* forced vital capacity, FEV_1 1st second forced expiratory volume, FEV_1/FVC ratio (absolute value in %), *MMEF* maximal mid expiratory flow, *MEFx%* maximal expiratory flow at x% of FVC (x = 25 or 50 or 75%), *FeNO* fraction of exhaled nitric oxide

*P value < 0.05: comparison of FeNO values between (5–17) and (17–25) ranges using t test

**P value < 0.05: comparison of FeNO values between (45–55) and (55–65) ranges using t test

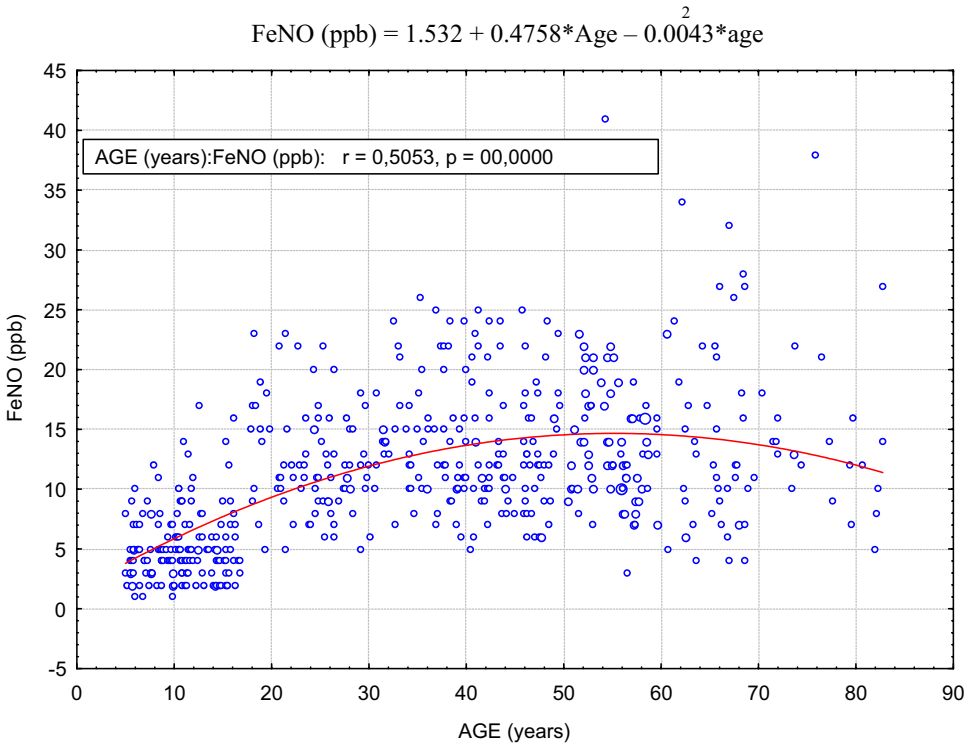

Fig. 1 FeNO values of the total sample according to age

The age from which there was a significant increase of FeNO value according to the age was at 13.97 years, 13.90 years, and 13.44 years for, respectively, the total sample, females, and males. So, the increase in the FeNO value was almost similar for both genders.

Twelve (aged over 50 years) patients had high FeNO values over 25 ppb, and 2 of them had FeNO values over 34 ppb. The majority of these patients (11 patients) had an overweight and a low socioeconomic level.

For the total sample, the age from which there was a drop of FeNO value is 63.66 years. The drop of FeNO values according to the age and gender were more precocious but not significant in elderly males than females with, respectively, breakpoints at 63.23 and 64.04 years.

Discussion

The FeNO value of a large group of North African healthy subjects aged 05–83 years (Arab race) from the region of Sousse was prospectively measured. A significant increase in FeNO is noted between groups with respective age ranges of (5, 17) and (17, 25) years with a breakpoint at 13.97 years. A significant decrease of FeNO is noted between groups with respective age ranges of (45, 55) and (55, 65) years with a breakpoint at 63.66 years. No statistical significant difference was found between females' and males' means FeNO data. Finally, SEL, obesity status, and hypertension contribute significantly in the variations of FeNO values.

Subject's Data

Sample

As for almost all the studies aiming to study the effect of age on functional or biological variables [3–11, 25–28], the present study has a convenience sample.

The recruitment modes are similar to previous studies having comparable aims to the present one [3–11, 18, 19, 28].

The subject age range selected **(05–83 years old)** was a combination of the data of three studies recently published about FeNO norms of Tunisian children [9], adults [10], and elderly [11].

An initial sample of 914 volunteer healthy subjects was examined. Non-inclusion criteria were found in 324 subjects. Thus, Five hundred and ninety (275 males) subjects were included to the present study.

Our sample size ($n = 590$ subjects) appears to be representative and enabled us to obtain reliable results. When it is compared with those of others studies which they specified a number of subjects similar of our range of age and have a comparative aims $n = 240$ [29]; $n = 193$ [7]; $n = 433$ [30], the present study sample seemed to be satisfactory.

Twelve (aged more than 50 years) patients had high FeNO values (over 25 ppb) which could be explained in part by the overweight and low socioeconomic level. Sfaxi et al. [11] showed that FeNO value between 25 and 34 can be considered as normal elderly patients.

Influencing Factors of FeNO Values Variations

Clinical Factors

The comparisons of FeNO values according to physical activity, instruction levels, and diabetes showed that FeNO levels were unaffected by these settings ($P > 0.05$). However, socioeconomic levels (SEL), and High blood pressure contributed significantly in FeNO modifications ($P < 0.05$).

Edith et al. showed that children with asthma presented an inverse association SEL with change in FeNO levels in response to the conflict task, meaning that as SEL declined, greater increases in FeNO were observed. This study suggested that lower SEL children with asthma may be more vulnerable to heightened airway inflammation in response to stress [31]. As demonstrated by Leng et al., low SEL is associated with higher blood pressure and so to higher FeNO levels [32].

Height, Weight, and Body Mass Index

There are a significant difference in anthropometric data (weight, height, and BMI) between males and females of the total sample. This result can be explained by the difference of hormonal status between genders. 128 patients are obese (93 females). A significant and positive correlation is found between FeNO and BMI values ($r = 0.125$). Indeed, obese patients had a significant higher values of FeNO than non-obese with respective FeNO mean values 13,740 ppb and 10.726 ppb ($P < 0.05$). A significant correlation ($P < 0.05$) was found between FeNO values and BMI ($r = 0.12$). Ciprandi et al. [33] showed that increased BMI did not affect FeNO values and asthma control level. In other studies [34, 35] in healthy subjects, without asthma, obesity/adiposity had a significant systemic inflammation derived from adipose tissue which did not affect eosinophilic airway inflammation (FeNO < 25 ppb).

Gender

No statistical significant difference was found between females' and males' means FeNO data of the total sample, with respectively, FeNO values 11.50 ± 6.06 ppb versus 11.22 ± 6.39 ppb ($P > 0.05$). In a large sample by many authors, no gender difference was found [3, 9, 30]. In other studies [7] a significant difference between genders was found. Matsunaga et al. [29] showed that the mean FeNO level for males was significantly higher than that for females, which is consistent with previous reports [36] but not with the present study.

Högman et al. [30] found that it was only in the middle age group where a gender difference could be found in FeNO values. Olin et al. found FeNO to be higher in men than in women around 50 years of age but when comparing FeNO between the sexes with similar heights and ages, no difference was found [3].

Age

The development and aging of the lung touched both respiratory and non-respiratory functions [19]. The lung matures by the age of 20 and age-related changes start around middle age, at 40–50 years. In the present study, FeNO has been shown to be age dependent. In the < 20 years age groups, FeNO was lower than in the other age groups (> 20 years).

Lung Development

The FeNO increase breakpoint appeared around 13.970 years for the total sample with no significant difference between genders. Development of the lung can be divided into two phases, lung growth (structural development) and lung maturation (functional development). Lung growth can be influenced by a host of physical factors. Lung maturation and the achievement of functionality is primarily a biochemical process and is under the control of a number of different hormones. Lung development finishes with adulthood [12].

In the under 20 year's age groups, FeNO was lower than in the other age groups. This could possibly reflect an increasing mucosal surface area with increasing height and growing lung volumes [9, 30]. This was also present in the study by Jacinto et al. where the FeNO increase breakpoint appeared around 14 years in girls and 16 years in boys [37]. This is in line with the growth of the body, and more specifically the development of the bronchial tree.

Aging

For the total sample, the age from which there is a drop of FeNO value is 63.66 years with no difference between genders. Indeed, lung aging is a determining factor in the alteration of respiratory functions [18, 19] but also of the non-respiratory functions such as inflammatory and immunology factors: NO production by the respiratory tract, oxidant–antioxidant balance [19]. It is well known that respiratory oxidative stress and inflammatory responses can be provoked by intrinsic (genetic) and extrinsic factors (smoking, air pollution…) [38, 39]. Sfaxi et al. showed that FeNO value is considered abnormal in elderly Tunisian population when it is of more than 34 ppb [11]. It is not clear why in elderly patients we did not observe any difference of FeNO levels by gender, but it is possible that, like other parameters, FeNO values also change after women's menopause becoming similar to men's levels [40].

In conclusion, we have demonstrate that the development and aging of the lung touched the bronchial

"inflammometer" and so modified FeNO values in healthy North African subjects (05–83 years). The FeNO increase breakpoint appeared around 13.970 years for the total sample with no significant difference between genders. It is in relation with the development of the bronchial tree. FeNO levels in healthy subjects over 65 years of age are influenced by intrinsic and extrinsic aging of the lungs.

Impact of this Research on Clinical Medicine and Basic Science

Recently, in North African population, the FeNO values of a large group of healthy children, adults, and elderly subjects were prospectively separately measured. It was shown that the available published children, adults, and elderly norms did not reliably predict FeNO values in these populations and that the three age ranges had different FeNO values. To determine and to explain the effect of age (development and aging) on FeNO values, we established this study. Physician had to adapt his care according to the values of FENO, taking into account lung development and aging.

Acknowledgements The authors thank Ines Sfaxi, Ashraf Alquodwa, Mohamed Ali Chouchene, and all the subjects who participated in the present study.

Compliance with Ethical Standards

Conflict of interest All authors declare that they have no conflicts of interest concerning this article.

References

1. Taylor DR (2006) Nitric oxide as a clinical guide for asthma management. J Allergy Clin Immunol 117(2):259–262
2. Bateman ED, Hurd SS, Barnes PJ, Bousquet J, Drazen JM, FitzGerald M et al (2008) Global strategy for asthma management and prevention: GINA executive summary. Eur Respir J 31(1):143–178
3. Olin A-C, Rosengren A, Thelle DS, Lissner L, Bake B, Torén K (2006) Height, age, and atopy are associated with fraction of exhaled nitric oxide in a large adult general population sample. Chest 130(5):1319–1325
4. Kovesi T, Kulka R, Dales R (2008) Exhaled nitric oxide concentration is affected by age, height, and race in healthy 9- to 12-year-old children. Chest 133(1):169–175
5. Olivieri M, Talamini G, Corradi M, Perbellini L, Mutti A, Tantucci C et al (2006) Reference values for exhaled nitric oxide (reveno) study. Respir Res 7:94
6. Dressel H, de la Motte D, Reichert J, Ochmann U, Petru R, Angerer P et al (2008) Exhaled nitric oxide: independent effects of atopy, smoking, respiratory tract infection, gender and height. Respir Med 102(7):962–969
7. Travers J, Marsh S, Aldington S, Williams M, Shirtcliffe P, Pritchard A et al (2007) Reference ranges for exhaled nitric oxide derived from a random community survey of adults. Am J Respir Crit Care Med 176(3):238–242
8. Kim S-H, Kim TH, Sohn JW, Yoon HJ, Shin DH, Park SS (2010) Reference values and determinants of exhaled nitric oxide in healthy Korean adults. J Asthma Off J Assoc Care Asthma 47(5):563–567
9. Rouatbi S, Alqodwa A, Ben Mdella S, Ben Saad H (2013) Fraction of exhaled nitric oxide (FeNO) norms in healthy North African children 5–16 years old. Pediatr Pulmonol 48(10):981–995
10. Rouatbi S, Chouchene MA, Sfaxi I, Rejeb MB, Tabka Z, Saad HB (2014) Fraction of exhaled nitric oxide (FeNO) norms in healthy Tunisian adults. BioMed Res Int 2014:269670
11. Sfaxi I, Saad HB, Rouatbi S (2015) Fraction of exhaled nitric oxide in healthy elderly Tunisian subjects. Nitric Oxide 50:88–97
12. Solaligue DES, Rodríguez-Castillo JA, Ahlbrecht K, Morty RE (2017) Recent advances in our understanding of the mechanisms of late lung development and bronchopulmonary dysplasia. Am J Physiol Lung Cell Mol Physiol 313(6):L1101–L1153
13. Schittny JC (2017) Development of the lung. Cell Tissue Res 367(3):427–444
14. Schmiedl A, Roolfs T, Tutdibi E, Gortner L, Monz D (2017) Influence of prenatal hypoxia and postnatal hyperoxia on morphologic lung maturation in mice. PloS ONE 12(4):e0175804
15. Morbidelli L, Chang CH, Douglas JG, Granger HJ, Ledda F, Ziche M (1996) Nitric oxide mediates mitogenic effect of VEGF on coronary venular endothelium. Am J Physiol 270:H411–H415
16. Ziche M, Morbidelli L, Choudhuri R, Zhang HT, Donnini S, Granger HJ, Bicknell R (1997) Nitric oxide synthase lies downstream from vascular endothelial growth factor-induced but not basic fibroblast growth factor-induced angiogenesis. J Clin Invest 99:2625–2634
17. The Official Workshop Report (2004) Mechanisms and limits of induced postnatal lung growth. Am J Respir Crit Care Med 170:319–343
18. Rouatbi S, Dardouri K, Farhat Ouahchi Y, Mdella SB, Tabka Z, Guenard H (2006) Aging of the lung parenchyma. Rev Mal Respir 23(5 Pt 1):445–452
19. Guénard H, Rouatbi S (2004) Physiological aspects of the decline of pulmonary function with age. Rev Mal Respir 21(5 Pt 3):8S13–8S24
20. Ferris BG (1978) Epidemiology standardization project (American Thoracic Society). Am Rev Respir Dis 118(6 Pt 2):1–120
21. Obesity (2000) preventing and managing the global epidemic. Report of a WHO consultation. World Health Organ Tech Rep Ser 894:1–253
22. American Thoracic Society, European Respiratory Society (2005) ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. Am J Respir Crit Care Med 171(8):912–930
23. Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R et al (2005) Interpretative strategies for lung function tests. Eur Respir J 26(5):948–968
24. Tabka Z, Hassayoune H, Guenard H, Zebidi A, Commenges D, Essabah H et al (1995) Spirometric reference values in a Tunisian population. Tunis Méd 73(2):125–131
25. Olin A-C, Bake B, Torén K (2007) Fraction of exhaled nitric oxide at 50 mL/s: reference values for adult lifelong never-smokers. Chest 131(6):1852–1856
26. Habib SS, Abba AA, Al-Zoghaibi MA, Subhan MM (2009) Reference range values of fractional exhaled nitric oxide in healthy Arab adult males. Saudi Med J 30(11):1395–1400
27. Gelb AF, George SC, Camacho F, Fraser C, Taylor CF, Shakkottai S (2011) Increased nitric oxide concentrations in the small airway of older normal subjects. Chest 139(2):368–375

28. Jo E-J, Song W-J, Kim T-W, Park H-W, Chang Y-S, Kim T-B et al (2014) Reference ranges and determinant factors for exhaled nitric oxide in a healthy korean elderly population. Allergy Asthma Immunol Res 6(6):504–510
29. Matsunaga K, Hirano T, Kawayama T, Tsuburai T, Nagase H, Aizawa H, Akiyama K, Ohta K, Ichinose M (2010) Reference ranges for exhaled nitric oxide fraction in healthy Japanese adult population. Allergol Int 59(4):363–367
30. Högman M, Thornadtsson A, Liv P, Hua-Huy T, Dinh-Xuan AT, Tufvesson E, Dressel H, Janson C, Koskela K, Oksa P, Sauni R, Uitti J, Moilanen E, Lehtimäki L (2017) Effects of growth and aging on the reference values of pulmonary nitric oxide dynamics in healthy subjects. J Breath Res 11(4):047103
31. Chen E, Strunk RC, Bacharier LB, Chan M, Miller GE (2010) Socioeconomic status associated with exhaled nitric oxide responses to acute stress in children with asthma. Brain Behav Immun 24(3):444–450
32. Leng B, Jin Y, Li G, Chen L, Jin N (2015) Socioeconomic status and hypertension: a meta-analysis. J Hypertens 33(2):221–229
33. Ciprandi G, Schiavetti I, Fontana RB, Sorbello V, Ricciardolo FL (2014) Overweight and obesity as risk factors for impaired lung function in patients with asthma: a real-life experience. Allergy Asthma Proc 35(4):e62–e71
34. Kim SH, Kim TH, Lee JS, Koo TY, Lee CB, Yoon HJ, Shin DH, Park SS, Sohn JW (2011) Adiposity, adipokines, and exhaled nitric oxide in healthy adults without asthma. J Asthma 48(2):177–182
35. Dweik RA, Boggs PB, Erzurum SC, Irvin CG, Leigh MW, Lundberg JO, Olin A-C, Plummer AL, Taylor DR (2011) An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (Fe_{NO}) for clinical applications. Am J Respir Crit Care Med 184(5):602–615
36. Travers J, Marsh S, Aldington S et al (2006) Reference values for exhaled nitric oxide (REVENO) study. Respir Res 7:94.
37. Jacinto T, Malinovschi A, Janson C, Fonseca J and Alving K (2015) Evolution of exhaled nitric oxide levels throughout development and aging of healthy humans. J Breath Res 9(3):036005
38. Bentayeb M, Billionnet C, Baiz N, Derbez M, Kirchner S, Annesi-Maesano I (2013) Higher prevalence of breathlessness in elderly exposed to indoor aldehydes and VOCs in a representative sample of French dwellings. Respir Med 107(10):1598–1607
39. Khansari N, Shakiba Y, Mahmoudi M. Chronic inflammation and oxidative stress as a major cause of age-related diseases and cancer. Recent Pat Inflamm Allergy Drug Discov 2009;3(1):73–80
40. Malerba M, Damiani G, Carpagnano GE, Olivini A, Radaeli A, Ragnoli B, Foschino MP, Olivieri M (2016) Values in elderly people for exhaled nitric oxide study. Rejuvenation Res 19(3):233–238