Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 269670, 13 pages
http://dx.doi.org/10.1155/2014/269670

Research Article

Fraction of Exhaled Nitric Oxide (Fe_{NO}) Norms in Healthy Tunisian Adults

Sonia Rouatbi,[1,2] Mohamed Ali Chouchene,[1] Ines Sfaxi,[1,2] Mohamed Ben Rejeb,[3] Zouhair Tabka,[1,2] and Helmi Ben Saad[1,2,4]

[1] *Department of Physiology and Functional Explorations, Farhat HACHED Hospital, 4000 Sousse, Tunisia*
[2] *Laboratory of Physiology, Faculty of Medicine of Sousse, University of Sousse, 4000 Sousse, Tunisia*
[3] *Department of Prevention and Care Safety, Sahloul Hospital, 4000 Sousse, Tunisia*
[4] *Research Unit: Secondary Prevention after Myocardial Infarction, N: 04/UR/08-18, Faculty of Medicine of Sousse, University of Sousse, 4000 Sousse, Tunisia*

Correspondence should be addressed to Sonia Rouatbi; sonia.rouatbi@rns.tn

Received 5 February 2014; Revised 23 April 2014; Accepted 23 April 2014; Published 3 June 2014

Academic Editor: Enrico Heffler

Copyright © 2014 Sonia Rouatbi et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Aims. To establish Fe_{NO} norms for healthy Tunisian adults aged 18–60 years and to prospectively assess their reliability. *Methods*. This was a cross-sectional analytical study. A convenience sample of healthy Tunisian adults was recruited. Subjects responded to a medical questionnaire, and then Fe_{NO} levels were measured by an online method (Medisoft, Sorinnes (Dinant), Belgium). Clinical, anthropometric, and plethysmographic data were collected. All analyses were performed on natural logarithm values of Fe_{NO}. *Results*. 257 adults (145 males) were retained. The proposed reference equation to predict Fe_{NO} value is $\ln Fe_{NO}$ (ppb) = 3.47−0.56× height (m). After the predicted Fe_{NO} value for a given adult was computed, the upper limit of normal could be obtained by adding 0.60 ppb. The mean ± SD (minimum-maximum) of Fe_{NO} (ppb) for the total sample was 13.54 ± 4.87 (5.00–26.00). For Tunisian and Arab adults of any age and height, any Fe_{NO} value greater than 26.00 ppb may be considered abnormal. Finally, in an additional group of adults prospectively assessed, we found no adult with a Fe_{NO} higher than 26.00 ppb. *Conclusion*. The present Fe_{NO} norms enrich the global repository of Fe_{NO} norms that the clinician can use to choose the most appropriate norms.

1. Introduction

The measurement of the fraction of nitric oxide in exhaled breath (Fe_{NO}) is recognized as an accurate, reproducible, and completely noninvasive diagnostic test for airway disease [1]. In 2011, the American Thoracic Society (ATS) recommended that measuring Fe_{NO} can help diagnose eosinophilic airway inflammation, determine the likelihood of corticosteroid responsiveness and the potential need for corticosteroids, unmask unsuspected nonadherence to corticosteroid therapy, and aid asthma assessment [2].

In health, the Fe_{NO} largely derives from the lower respiratory tract, particularly the airways of the lung, if nasal air is excluded [3]. NO can be detected in exhaled air by several methods such as chemiluminescence, spectroscopy, electrochemical portable, and other methods currently under development [4]. Cheaper and easy to use [1], Fe_{NO} analyzers are now readily available and increasingly used not only for the diagnosis of eosinophilic airway inflammation which is seen mainly in asthma [5] but also for its assessment [6]. In addition, the ATS/European Respiratory Society (ATS/ERS) has jointly demonstrated that some factors (i.e., age, sex, and race) may affect the Fe_{NO} values [1].

Interpretation of Fe_{NO} data relies upon comparison of measured values with predicted ones available from published norms (e.g., fixed values, reference equations or normal values tables) [1, 7, 8]. However, to the best of our knowledge, Fe_{NO} norms are available only for some adult populations, mainly for Caucasians ones [9–24]. These norms can be used in clinical practice, provided that the study characteristics (population, sampling, and objective measures) are taken into consideration when such an equation is used for the interpretation of Fe_{NO} values [1, 7]. The published norms [9–24] differ considerably in terms of individual-specific

factors that have an effect on Fe_{NO} values, and there is little standardization of the method description in the studies, both on the statistical and technical sides [7]. In addition, neither of these studies provided prospective verification for their studied populations nor proposed a clear method of interpreting the measured Fe_{NO} (e.g., using an upper-limit-of-normal (ULN) or a fixed percentage above which Fe_{NO} values would be considered abnormal). In addition, only few studies have included a comparison group [12, 13, 18, 21].

Recently, the Fe_{NO} of a large group of healthy Tunisian/Arab children was prospectively measured [25]. It was shown that the available published children Fe_{NO} norms did not reliably predict Fe_{NO} in this population [25]. Thus, a table of normal values according to age ranges was established. In addition, the need of reference equations specific to Tunisian/Arab adults' populations has been demonstrated for several lung function parameters [26–33], but not for Fe_{NO}. Furthermore, and to the best of our knowledge, Fe_{NO} norms are established only in one Arabic population (Saudi Arabian males' [16]) and the applicability and reliability of these norms [16] should be assessed as regards to Tunisian Arab adults, in order to avoid erroneous clinical interpretation of Fe_{NO} data in this population. Moreover, the ATS/ERS has encouraged investigators to publish physiological norms for healthy populations of various racial backgrounds to enable individual subject results to be compared with data from a racially similar population [1]. The use of the same kind of assessment equipment and procedure is also recommended [1]. Therefore, the present study aims

(1) to identify factors that influence the Fe_{NO} values of healthy Tunisian adults aged 18–60 years,

(2) to test the applicability and reliability of the previously published Fe_{NO} norms for Saudi Arabian males [16] (the null hypothesis is that there will be no difference between measured and predicted Fe_{NO} mean values),

(3) if needed to establish Fe_{NO} norms and to prospectively assess their reliability.

2. Methods

2.1. Study Design. The present study is a cross-sectional one spread over 7 months (May–December 2012). It was conducted at the Department of Physiology and Functional Explorations (Farhat HACHED Hospital, Sousse, Tunisia).

Study design consists of a convenience sample of healthy Tunisian adults aged 18–60 years (Arab race) in the region of Sousse.

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

Detailed information about the study design appears in the Supplemental Data available online at http://dx.doi.org/10.1155/2014/269670.

2.2. Sample Size. It was calculated according to the following predictive equation [34]: $n = (Z^2Pq)/\Delta^2$, where "n" was the number of required adult, "Z" was the 95% confidence level (=1.96), "q" was equal to "$1 - P$", "Δ" was the precision (= 6%), and "P" was the estimation of adults aged more than 18 years with a normal Fe_{NO} value. Among the 298 participants (aged 25–75 years) who performed Fe_{NO} measurements, only 193 adults ($P = 0.65$) were categorized as normal by Travers et al. [13]. Plugging this relevant value into the predictive equation, the sample size was thus 243 adults. Therefore, to establish Fe_{NO} norms, we recruited an initial group (*equation group*) of 257 adults (145 females).

To verify the reliability of the present study norms, Fe_{NO} data were prospectively measured in a second group (*validation group*) of 50 additional healthy adults (25 females) meeting the inclusion criteria of the present study but not having participated in the first part.

2.3. Subjects. Volunteer healthy adults were included.

The following noninclusion criteria were applied: hay fever or chronic illnesses especially cardiovascular, renal, gastrointestinal, or neurological diseases; otorhinolaryngologic diseases or symptoms (allergic rhinitis, recurrent symptoms or rhinitis, symptoms and signs of acute upper respiratory infection during two weeks prior to assessment, and recent airway infection (cold, flu, and sore throat within the last seven days)); clinical manifestation of allergic diseases (urticaria, skin allergy, atopic dermatitis, or eczema); a history of pulmonary diseases or related respiratory symptoms (history of asthma or asthma medication use, current or past symptoms of wheeze or chronic cough, and chronic obstructive pulmonary disease); abnormal lung function data; pregnant at the time of assessment; regular medication (glucocorticoid, bronchodilator, leukotriene receptor agonist, antihistamine, etc.) use except contraceptive; current or ex-smokers (cigarettes or narghile use [35, 36]) and inability to perform properly Fe_{NO} or plethysmography measurements.

2.4. Medical Questionnaire and Physical Examination. A medical questionnaire [37] was used to assess several subject characteristics.

Age (yrs) was taken as the number of complete years from birth to the date of the study. Height (±0.01 m) and weight (±1 kg) were measured with a height gauge with shoes removed, heels joined, and back straight and subject without heavy clothes. Body mass index (BMI) was calculated (= weight/height^2). Two groups of subjects were defined [38] nonobese (BMI < 30); obese (BMI ≥ 30). Body surface area (BSA, m^2) was calculated [39].

2.4.1. Fe_{NO} Measurement. The Fe_{NO} (parts per billion, ppb) was measured by Medisoft HypAir Fe_{NO} 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 [1]. After a full unforced exhalation outside the mouthpiece,

a maximal inspiration was performed through an absorber to ensure NO-free air. The adult then performed a controlled exhalation using flow control at an exhalation pressure of 4–10 cm H_2O for at least six seconds, during which time sample collection and gas analysis were performed. Nasal contamination is presented by closure of the velum by using five cm H_2O oral back pressures. 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 two hours prior to the test [1].

Three acceptable measurements (within 10%) were taken at the recommended flow rate of 50 mL/s within a 15-minute period [1].

2.4.2. Plethysmography Measurements. They were performed according to international guidelines [40] using a plethysmograph (ZAN 500, Meβgreräte GmbH, Germany). Tests were made after the Fe_{NO} measurement [41].

The following parameters were measured/calculated: peak expiratory flow (PEF); forced vital capacity (FVC, L); 1st second forced expiratory volume (FEV_1, L); maximal mid expiratory flow (MMEF, L/s) or forced expiratory flow when x% of FVC has been exhaled (MEFx, L/s); FEV_1/FVC ratio (absolute value); total lung capacity (TLC, L); residual volume (RV, L); and thoracic gas volume (TGV, L). The results were compared with local age- and sex-matched reference values [42].

Obstructive or restrictive ventilatory defects were retained when, respectively, the FEV_1/FVC ratio or the TLC was lower than the lower limit of normal (LLN) [40]. FEV_1 and FVC were considered as abnormal when they were lower than the LLN [40].

2.5. Statistical Analysis. For each subject, the mean of the three correct Fe_{NO} values was used for statistical analysis.

Preliminary descriptive analysis included frequencies for categorical variables (sex: male/female) and obesity status (nonobese/obese) and means ± standard deviation (SD) and 95% confidence interval (95% CI) for continuous ones (anthropometric and plethysmographic data).

Since the distribution of the dependent variable (Fe_{NO}) was log-normally distributed (Shapiro-Wilk test [43]), all analyses were performed on natural logarithm values of Fe_{NO} ($\ln Fe_{NO}$). Fe_{NO} results were presented as geometric mean ± SD (95% CI, LLN to ULN) and as minimum-maximum.

Comparison with Published Fe_{NO} Norms for Saudi Arabian Males [16]. Habib et al. [16] developed two linear models (Box 1) for 121 Saudi Arabian males aged 19–64 years. More details about these norms are exposed in Supplemental Table 1. Individually measured present study males' Fe_{NO} was compared with the predicted Fe_{NO} from the two reference equations [16] for the same age range, using paired t-tests and scatter plots. Limits of agreement (measured-predicted) were calculated. If the Saudi Arabian reference equations provide limits of agreement closest to zero, they will be appropriate for the present population [40].

Model 1. Fe_{NO} (ppb) = 47.096 − 0.119 × Weight (kg)
Model 2. Fe_{NO} (ppb) = 31.541 − 0.289 × BMI (kg/m^2)

Box 1: Fe_{NO} norms for Saudi Arabian males [16].

It is well known that Fe_{NO} values obtained with different devices are not directly comparable [44]. As the Aerocrine devices are much more commonly used and most of the other devices give pretty similar results [44] and as measurements on the HypAir Fe_{NO} are 1.6 times higher than those obtained with the Aerocrine NIOX [45] and for a better interpretation of the present study data, results were adjusted in accordance with Brooks et al. [45]. For that reason Fe_{NO} predicted values from Habib et al. [16] norms were divided by 1.6 and individually measured Fe_{NO} were compared with the predicted/adjusted Fe_{NO} from Habib et al. [16] norms as described above.

Student's t-tests were used to evaluate the associations between Fe_{NO} and the categorical variables. Pearson product-moment correlation coefficients evaluated the associations between Fe_{NO} and the continuous measures. The linearity of association between Fe_{NO} and the continuous measures was checked graphically by plotting each regressor against the Fe_{NO}. Only significantly and linearly associated variables were entered into the model. A linear regression model was used to evaluate the independent variables explaining the variance in Fe_{NO}. Candidate variables were stepped into the model with a stepwise selection method. To determine entry and removal from the model, significance levels of 0.15 and 0.05 were used, respectively. No colinearity between predictors was detected with variance inflation factors. The linearity was evaluated by correlation (r) and determination (r^2) coefficients and the standard error. The 95% CI (= 1.64 × residual-SD (RSD)) was calculated [43]. Detailed information about the natural logarithm linear regression equation appears in the Supplemental Data.

Fe_{NO} Reference Equations and Normal Values. Three Fe_{NO} reference equations (for males, females, and total sample) were established, using only previously correlated factors in a stepwise linear regression model. A measured Fe_{NO} higher than the ULN (ULN = reference value + 1.64 × RSD) will be considered as abnormal.

A table for each age and height ranges for the total sample, presenting Fe_{NO} geometric mean ± SD (95% CI, LLN to ULN and minimum-maximum) is provided. Three ways are proposed to interpret a measured Fe_{NO} value.

(i) Use of the total sample Fe_{NO} maximum value as a threshold: each adult Fe_{NO} value higher than the total sample Fe_{NO} maximum value will be considered as abnormal.

(ii) Use of a specific threshold (Fe_{NO} maximum value) for each age or height ranges: each Fe_{NO} value higher than

Table 1: Healthy Arab Tunisian never-smoking adults' characteristics.

		Females (n = 145)	Males (n = 112)	Total sample (n = 257)
Anthropometric data (data are mean ± SD)				
Age	(year)	38.04 ± 11.55	41.09 ± 11.43*	39.37 ± 11.58
Weight	(kg)	70 ± 13	79 ± 14*	74 ± 14
Height	(m)	1.60 ± 0.06	1.73 ± 0.07*	1.65 ± 0.09
Body mass index	($kg{\cdot}m^{-2}$)	27 ± 5	26 ± 4	27 ± 5
Body surface area	(m^2)	1.72 ± 0.15	1.92 ± 0.18*	1.81 ± 0.19
Plethysmographic and fraction-of-exhaled-nitric-oxide (Fe_{NO}) data (data are mean ± SD)				
FVC	(%)	99 ± 12	95 ± 11*	97 ± 12
FEV_1	(%)	96 ± 11	93 ± 11*	94 ± 11
PEF	(%)	78 ± 14	79 ± 14	79 ± 14
MMEF	(%)	89 ± 21	82 ± 18*	86 ± 20
MEF_{25}	(%)	74 ± 29	73 ± 34	74 ± 31
MEF_{50}	(%)	85 ± 20	87 ± 20	86 ± 20
MEF_{75}	(%)	82 ± 15	86 ± 16	84 ± 16
TLC	(%)	96 ± 11	92 ± 12*	94 ± 12
TGV	(%)	98 ± 20	105 ± 28*	101 ± 24
RV	(%)	101 ± 32	101 ± 37	101 ± 34
Fe_{NO}	(ppb)	13.31 ± 4.55	13.84 ± 5.26	13.54 ± 4.87
$lnFe_{NO}$	(ppb)	2.53 ± 0.35	2.55 ± 0.40	2.54 ± 0.37
Obesity status (data are number (%))				
Obesity status	Normal weight	51 (35%)	44 (39%)	95 (37%)
	Overweight	49 (34%)	47 (42%)	96 (37%)
	Obesity	45 (31%)	21 (19%)**	66 (26%)

For abbreviations, ln: natural logarithm.
Plethysmographic data are expressed as percentage (%) of predicted value.
* $P < 0.05$ (Mann-Whitney U-test): females versus males.
** $P < 0.05$ (chi-2): females versus males.

these ages or height ranges Fe_{NO} maximum values will be considered as abnormal.

(iii) Use of a specific threshold (Fe_{NO} maximum value) for each age and height range: each Fe_{NO} value higher than this age and height range Fe_{NO} maximum value will be considered as abnormal.

Reliability of the Arab Tunisian Fe_{NO} Norms. It was evaluated in the validation group in two ways. Fe_{NO} predicted normal values will be considered as reliable when no subject from the validation group will have a measured abnormal Fe_{NO} value (higher than predicted Fe_{NO} maximum value for each age and height ranges). The correlation between the measured Fe_{NO} values and those predicted by the Fe_{NO} reference equations is evaluated. The number of subjects having a measured Fe_{NO} value higher than the ULN is determined.

Analyses were carried out using Statistica (Statistica Kernel version 6, StatSoft, 26 France). Significance was set at the 0.05 level.

3. Results

3.1. Subject's Data. An initial sample of 400 voluntary adults of Arab race was examined. Noninclusion criteria, presented in detail in the Supplemental Data, were found in 93 subjects.

Two hundred and fifty-seven adults (equation group) were included to establish Fe_{NO} norms and 50 adults were included as a validation group.

Between the males and females of the equation group (Table 1 and Supplemental Table 3), there was a significant difference in anthropometric data (age, weight, height, and BSA) and plethysmographic data expressed in absolute values (exposed in Supplemental Table 3) (FVC, FEV_1, FEV_1/FVC, PEF, MMEF, MEF_{50}, MEF_{75}, TLC, TGV, and RV) or expressed as a percentage of predicted values (FVC, FEV_1, MMEF, TLC, and TGV). In addition, significantly higher females were categorized as obese. No statistical significant difference was found between females' and males' means Fe_{NO} data, respectively, 13.31 ± 4.55 versus 13.84 ± 5.26 ppb.

Supplemental Figure 1 shows the distribution of the 257 healthy adults according to sex and age range. Compared to females, there was a significantly lower number of males aged 17–35 years and a significantly higher number of males aged 45–55 years.

Supplemental Figure 2 shows the distribution of the adults Fe_{NO} data according to age, height, and weight ranges. A significant Fe_{NO} difference was found between subjects at the height of 1.36–1.55 m.

Table 2: Univariate analysis between the fraction-of-exhaled-nitric-oxide (Fe_{NO}) and healthy Arab Tunisian never-smoking adults' data.

		Females (n = 145)	Males (n = 112)	Total sample (n = 257)
Univariate analysis between Fe_{NO} data and continuous measures				
Age	(yr)	0.08	0.01	0.05
Weight	(kg)	−0.04	0.04	0.02
Height	(m)	−0.30*	−0.18*	−0.13*
Body mass index	($kg \cdot m^{-2}$)	0.08	0.14	0.10
Body surface area	(m^2)	−0.13	−0.02	−0.03
FVC	(L)	−0.23*	−0.09	−0.07
FVC	(%)	−0.03	0.01	−0.02
FEV_1	(L)	−0.24*	−0.09	−0.09
FEV_1	(%)	−0.06	−0.01	−0.04
FEV_1/FVC	(Absolute value)	−0.09	−0.01	−0.06
PEF	(L/s)	−0.22*	0.08	−0.01
PEF	(%)	−0.14	0.13	−0.01
MMEF	(L/s)	−0.21*	−0.04	−0.10
MMEF	(%)	−0.18*	−0.02	−0.12
MEF_{25}	(L/s)	−0.17*	−0.03	−0.09
MEF_{25}	(%)	−0.13	0.02	−0.05
MEF_{50}	(L/s)	−0.23*	−0.06	−0.11
MEF_{50}	(%)	−0.19*	−0.03	−0.11
MEF_{75}	(L/s)	−0.22*	0.06	−0.01
MEF_{75}	(%)	−0.17*	0.11	−0.02
TLC	(L)	−0.19*	−0.16	−0.08
TLC	(%)	−0.01	−0.09	−0.06
TVG	(L)	−0.20*	−0.15	−0.11
TVG	(%)	−0.14	−0.11	−0.12*
RV	(L)	−0.07	−0.17	−0.10
RV	(%)	−0.03	−0.15	−0.09
Univariate analysis between Fe_{NO} data and obesity status				
Obesity status	Normal weight or overweight	13.02 ± 4.52	13.87 ± 5.15	13.42 ± 4.84
	Obesity	13.96 ± 4.58	13.71 ± 5.87	13.88 ± 4.98

For abbreviations, see abbreviations list.
*$P < 0.05$ (univariate Spearman correlation coefficients between Fe_{NO} data and continuous measures).
**$P < 0.05$ (t-tests): females versus males.

3.2. Univariate Analysis. Sex (Table 1) and obesity status (Table 2) did not significantly affect the Fe_{NO} value.

For the total sample, Fe_{NO} was significantly correlated with height and TGV (%). For males, Fe_{NO} was significantly correlated only with height. For females, Fe_{NO} was significantly correlated with height and some plethysmographic data (FVC (L), FEV_1 (L), PEF (L/s), MMEF (L/s, %), MEF_{25} (L/s), MEF_{50} (L/s, %), MEF_{75} (L/s, %), and TGV (L)) (Table 2).

3.3. Multivariate Analysis (Fe_{NO} Influencing Factors, Table 3). For females, height (m), MEF_{50} (%), and TGV (L) explained a slight (r^2 = 9.24%) but significant Fe_{NO} variability. For males and the total sample, only height (m) explained a slight (resp., r^2 = 3.80% and r^2 = 1.92%) but significant Fe_{NO} variability. The retained Fe_{NO} reference equation is exposed in Box 2.

$$\ln Fe_{NO}\ (\text{ppb}) = 3.466936 - 0.560725 \times \text{Height (m)}$$

Box 2: Retained Fe_{NO} reference equation.

3.4. Comparison, without Values Adjustment according to Brooks et al. [45], with Published Fe_{NO} Norms for Saudi Arabian Males [16]. Figure 1 shows individually measured Fe_{NO} plotted against the corresponding predicted value for the same age range, using the Saudi Arabian model 1

Table 3: Independent variables included in the forward linear stepwise multiple regression model for the natural logarithm of fraction-of-exhaled-nitric-oxide (Fe_{NO}).

Independent variables	Nonstandardized regression coefficient (B)	95% confidence interval around each B	Cumulative determination coefficient (r^2)	P level	Standard error	1.64 residual standard deviation
			Females (n = 145)			
Constant	4.73424	3.48017 to 5.98832		0.000000		
Height (m)	−1.17043	−2.00733 to −0.33353	0.0685	0.023293	0.7534	0.54553
MEF_{50} (%)	−0.00209	−0.00446 to 0.00028	0.0839	0.149905	0.7510	
TGV (L)	−0.06149	−0.14921 to 0.02623	**0.0924**	0.252243	0.7647	
			Males (n = 112)			
Constant	4.47053	2.95938 to 5.98168		0.000004		0.641486
Height (m)	−1.11099	−1.98559 to −0.23639	**0.0380**	0.039545	0.9214	
			Total sample (n = 257)			
Constant	3.466936	2.78519 to 4.14868		0.000000		0.5992724
Height (m)	−0.560725	−0.97258 to −0.14887	**0.0192**	0.026431	0.41569	

For abbreviations, see abbreviations list.
For females: $\ln Fe_{NO}$ (ppb) = 4.73424 − 1.17043 × height (m) − 0.00209 × MEF_{50} (%) − 0.06149 × TGV (L).
For males: $\ln Fe_{NO}$ (ppb) = 4.47053 − 1.11099 × height (m).
For the total sample: $\ln Fe_{NO}$ (ppb) = 3.466936 − 0.560725 × height (m).

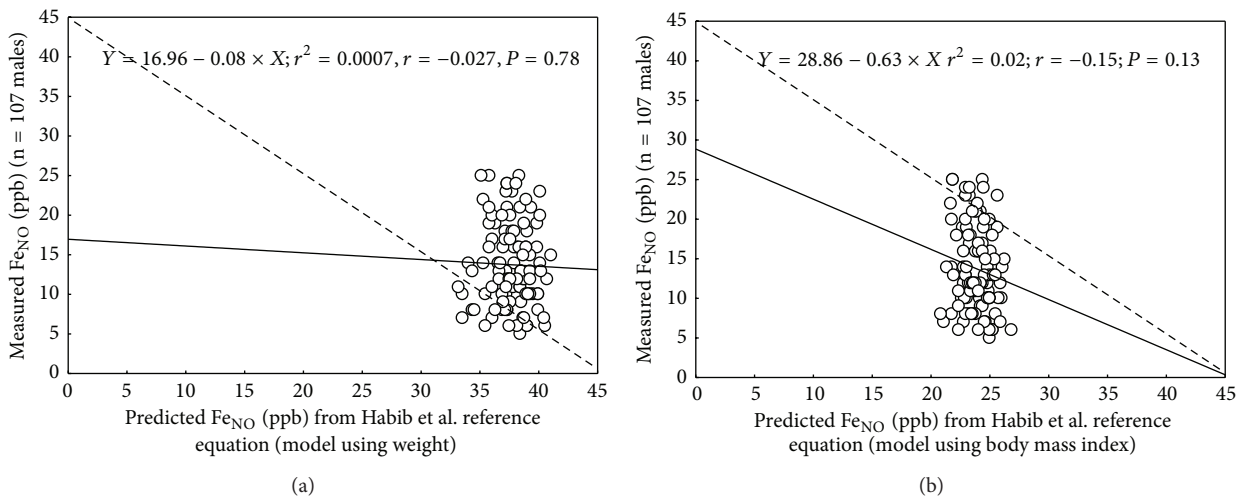


Figure 1: Comparison, for the same age range, of measured and predicted fraction-of-exhaled-nitric-oxide (Fe_{NO}) determined from Saudi Arabian norms: (a) model including weight. (b) Model including body mass index. n = number of males having the age range of the Saudi Arabian predicted Fe_{NO} study. Solid line (—): regression line. Dashed line (- - -): identity line. r^2: coefficient of determination. r: correlation coefficient. P: probability.

(Figure 1(a)) or model 2 (Figure 1(b)) reference equations. As can be seen, the data showed wide disparity compared to the identity line with a systematic bias between the measured and predicted values. In addition, the present study mean ± SD measured Fe_{NO} was significantly overestimated by 23.95 ± 5.58 ppb and by 10.12 ± 5.60 ppb, with, respectively, the model 1 (Figure 1(a)) and the model 2 (Figure 1(b)) reference equations.

3.5. Comparison, after Values Adjustment according to Brooks et al. [45], with Published Fe_{NO} Norms for Saudi Arabian Males [16]. Supplemental Figure 3 shows individually measured Fe_{NO} plotted against the corresponding predicted/adjusted value for the same age range, using the Saudi Arabian model 1 (Supplemental Figure 3(a)) or model 2 (Supplemental Figure 3(b)) reference equations. The data still showed disparity compared to the identity line with a systematic bias between the measured and predicted/adjusted values. The present study mean ± SD measured Fe_{NO} was significantly overestimated by 9.82 ± 5.41 ppb ($P < 0.05$) and only by 1.18 ± 5.45 ppb ($P = 0.02$), with, respectively, the model 1 (Supplemental Figure 3(a)) and the model 2 (Supplemental Figure 3(b)) reference equations.

3.6. Tunisian Adults Fe_{NO} Norms (Fe_{NO} Reference Equation or Table Norms). Due to the inadequacy of the Saudi Arabian males' Fe_{NO} reference equations [16], norms adapted to Tunisian population were established.

Table 4: Fraction-of-exhaled-nitric-oxide (Fe_{NO}) norms: Fe_{NO} data (ppb) according to ranges of height and age among 257 healthy Arab Tunisian never-smoking adults.

Height ranges (m)	Age ranges (year)				
	[17–35[	[35–45[	[45–55[	≥55	All ranges of age
[1.36–1.55[	16 ± 6 [9–**22**] (n = 5)	15 ± 5 [5–**24**] (n = 10)	15 ± 3 [12–**20**] (n = 8)	13 ± 6 [7–**21**] (n = 4)	15 ± 5 [5–**24**] (n = 27)
[1.55–1.65[	12 ± 5 [5–**24**] (n = 41)	13 ± 5 [7–**26**] (n = 29)	14 ± 4 [8–**22**] (n = 25)	11 ± 5 [6–**23**] (n = 8)	13 ± 5 [5–**26**] (n = 103)
[1.65–1.75[	12 ± 3 [7–**21**] (n = 27)	13 ± 6 [7–**25**] (n = 27)	15 ± 5 [8–**25**] (n = 18)	11 ± 7 [7–**19**] (n = 9)	13 ± 5 [7–**25**] (n = 81)
≥1.75	12 ± 5 [5–**23**] (n = 17)	14 ± 6 [6–**24**] (n = 10)	11 ± 5 [6–**21**] (n = 17)	12 ± 2 [11–**14**] (n = 2)	12 ± 5 [5–**24**] (n = 46)
All ranges of height	12 ± 4 [5–**24**] (n = 90)	13 ± 5 [5–**26**] (n = 76)	13 ± 5 [6–**25**] (n = 68)	11 ± 5 [6–**23**] (n = 23)	13 ± 5 [5–**26**] (n = 257)

Data are geometric mean ± standard deviation [minimum–maximum].
n = number of adults in each range.
Algorithm of interpretation:
Step 1. Determine each adult age and height ranges.
Step 2. Note, for these ranges, the Fe_{NO} maximum value (values in bold character).
Step 3. The measured Fe_{NO} is considered as abnormal when it is higher than the predicted Fe_{NO} maximum value previously determined.

For a practical interest, and as sex did not significantly affect the Fe_{NO} value, authors recommend the use of the total sample reference equation (Box 2), when calculating a predicted Fe_{NO} value. The latter explains almost 2% of the Fe_{NO} variability. After the predicted Fe_{NO} value for a given adult was computed from this equation, the ULN could be obtained by adding 0.5992724 ppb.

Since the correlation between height and Fe_{NO} was very slight, Fe_{NO} normal values for Arab Tunisian adults aged 18–60 years were developed, taking into consideration age and height ranges. These Fe_{NO} normal values are presented as geometric mean ± SD and minimum-maximum (Table 4). It is much simpler for clinicians to remember and device manufacturers to program. In practice, three ways can be used to interpret a measured Fe_{NO} value.

(i) Use of the total sample Fe_{NO} maximum value as a threshold: each adult Fe_{NO} value higher than 26 ppb will be considered as abnormal.

(ii) Use of a specific threshold (Fe_{NO} maximum value) for each age or height range: for example, for a given adult aged 17–35 years each Fe_{NO} value higher than 24 ppb will be considered as abnormal and for a given adult having a height range from 1.36 to 1.55 m, each Fe_{NO} value higher than 24 ppb will be considered as abnormal.

(iii) Use of a specific threshold (Fe_{NO} maximum value) for each age and height range: for example, for a given adult aged 17–35 years having a height range from 1.36 to 1.55 m, each Fe_{NO} value higher than 22 ppb will be considered as abnormal.

3.7. Reliability of Tunisian Fe_{NO} Norms. The mean ± SD age, height, weight, and BMI of the validity group were, respectively, 40.88 ± 13.45 years, 1.66 ± 0.09 m, 75 ± 22 kg, and 28 ± 9 kg/m^2. The validation group anthropometric data are similar to those of the equation (Supplemental Table 4). However, significant differences were noted for FVC, FEV_1 and RV expressed as percentages of predicted values (Supplemental Table 4). Supplemental Figure 4 exposes the measured Fe_{NO} values of the equation and validation groups according to height. The validation group Fe_{NO} values are closer to those of the equation.

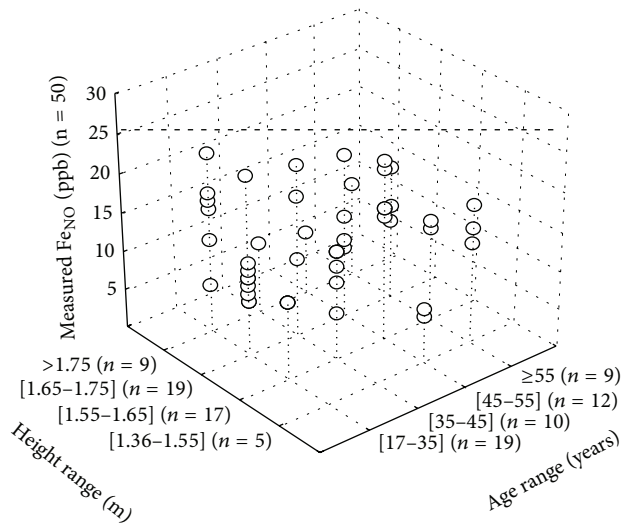


Figure 2: Three-dimension presentation (XYZ scatterplot) of the validity group measured fraction-of-exhaled-nitric-oxide (Fe_{NO}) values. Fe_{NO} (Y-axis) versus age (X-axis) and height (Z-axis) ranges described in Table 4. n = number of healthy Arab Tunisian subjects. Dashed line (- - -): predicted Fe_{NO} maximum value for the total sample (=26 ppb).

The validation group measured Fe_{NO} values are shown in Figure 2. The application of the normal values mentioned in

Table 4 found no adult with a measured Fe_{NO} higher than the predicted specific threshold for each age and height range. In addition, no adult had a measured Fe_{NO} value higher than the predicted total sample Fe_{NO} maximum value (=26 ppb).

The geometric mean ± SD (minimum-maximum) Fe_{NO} prospectively measured was 12 ± 5 (6–23) ppb. When expressed as a percentage of predicted value derived from the total sample reference equation (Box 2), the geometric mean ± SD (minimum-maximum) of the Fe_{NO} was 96 ± 39% (44–203).

4. Discussion

The Fe_{NO} of a large group of healthy Tunisian/Arab adults aged 18–60 years old was prospectively measured. The Fe_{NO} norms for Saudi Arabian did not reliably predict Fe_{NO} in the local population and Fe_{NO} values are lower in healthy Tunisian/Arab adults than in Saudi Arabian population. So, the null hypothesis that we would see no difference in the means of the measured and predicted Fe_{NO} mean values was rejected. Thus, a table of normal values according to age and height ranges was established. For Arab Tunisian adults of any age and height, any Fe_{NO} value greater than 26 ppb may be considered abnormal. In addition a reference equation taking into consideration height was established. Finally, in an additional group of 50 adults prospectively assessed, no adult with a Fe_{NO} higher than the threshold of 26 ppb or higher than the 95% CI ULN specific for each age and height ranges was found.

4.1. Subject's Data. As for almost all the studies aiming to publish Fe_{NO} norms [9–23] the present study was a convenience sample.

The recruitment mode and adult age range were similar to previous studies having comparable aims to the present one [9, 10, 12, 14, 16, 20, 22].

The present study which calculated sample size (n = 257) "seemed" to be satisfactory. The retained Fe_{NO} reference equation allowed the explanation of 2% of the Fe_{NO} variability, which appears to be less than reported data (r^2 ranged 6% [22] to 34% [10]).

Fe_{NO} was prospectively measured in a validation group of additional healthy adults meeting the inclusion criteria of the present study. To the best of our knowledge, the present study is the first one that uses a validation healthy group to verify the reliability of the retained Fe_{NO} norms.

Similar to some studies [9, 10, 15, 17, 18, 22], atopy was assessed using only questionnaires. However, it was preferable to determine the serum level of total immunoglobulin E (IgE) [11, 12, 16] or of specific IgE [11] or of eosinophil [12] or to estimate sensitization to allergens with prick testing [12, 13].

There are few studies [11, 20] that assessed the reference equations of Fe_{NO} in healthy adults nonsmoking adults, as in the present study. As in most studies [9, 11, 13, 16–18, 22], smoking status was subjectively assessed via the medical questionnaire. It was preferable to objectively assess it, for example, via serum cotinine levels [15].

The present study noninclusion criteria were similar to those applied in similar studies [9–24]. Obesity was present in 26% of the total samples. The included group composition reflected a "healthy" population, since 28% of the general local population over 20 years showed obesity [46]. In addition, as did some other authors, adults having obesity were not excluded [9–13, 15–18, 22].

To our knowledge, the present study is the first that measured lung volumes, which are important data for the diagnosis of restrictive defect and lung hyperinflation [27, 47].

Detailed discussion of the subject's data appears in the Supplemental Data.

4.2. Fe_{NO} Measurement. As in some studies [17, 19, 24], an electrochemical analyzer (Medisoft) was used. The majority of studies concerning adult Fe_{NO} norms [9–16, 18, 20–23] have used the chemiluminescence analyzers.

Because environmental NO can reach high levels relative to those in exhaled breath, standardized techniques must prevent the contamination of biological samples with ambient NO [1]. As recommended [1] notwithstanding which technique is used, ambient NO at the time of each test should be recorded. In the present study, mean ± SD (minimum-maximum) ambient NO concentration was 1.4 ± 1.4 ppb (0–5 ppb). Medisoft device has an absorption column with high capacities for detecting and eliminating ambient NO. Thus its function is not limited by the values of ambient NO.

Because plethysmographic maneuvers transiently reduce the Fe_{NO} levels [1], NO analysis was performed before plethysmography.

As measurements need to be standardized for time of day (circadian rhythm effects [21]), Fe_{NO} measurements were performed in the same period of the day.

4.3. Statistical Analysis. The dependent variable (Fe_{NO}) was logarithmically transformed in natural logarithm, as published elsewhere [10, 22].

The absolute values of Fe_{NO} were presented as geometric mean ± SD (95% CI, LLN, and ULN) and as minimum-maximum. In other studies [9–24], absolute values of Fe_{NO} were presented with great heterogeneity using several central tendency and dispersion measures (mean, geometric mean, median, interquartile range, SD, and 95% and 90% CIs) and in different subgroups.

Similar to other studies [11, 12], Fe_{NO} norms were presented in two ways: total sample reference equation and a table of limit values. In literature, Fe_{NO} norms were presented as reference equations [11, 12, 14, 16, 20–22, 24], as fixed threshold [9, 17], and as tables of limit values [10–13, 18, 19, 23].

Fe_{NO} reference norms should be further refined in the future [7], perhaps in ways similar to those recently reviewed for lung function measurements [48]. For example, samples with a wider range of ages and different races or ethnicities, multicentre research teams, and the use of standardized technical and statistical procedures are desirable features for Fe_{NO} norms studies [7].

4.4. Non-Disease-Related Subject Factors Influencing Fe_{NO} Values. Interpretation of Fe_{NO} values relies upon comparison with predicted values available from published norms [9–24]. To our knowledge, the present study is the first that reported Fe_{NO} norms for healthy Arab Tunisian adults. Therefore, there is a continuing need for such clinical research.

The multivariate analysis showed that height significantly affects the Fe_{NO} data. In addition, females Fe_{NO} data were significantly affected by MEF_{50} and TGV. These factors will be analyzed in the following sections.

Height Effect. Like other studies [11, 14, 20, 21, 24] the present one reported an association between height and Fe_{NO}. The origin in the airway epithelium indicates that the total surface area of the airway mucosa will be an important determinant for Fe_{NO} [7]. Indeed, the airway diffusing capacity for NO, which theoretically should be dependent on the airway mucosal surface area, has been shown to correlate with anatomic dead space volume in healthy subjects [49]. It is logical that height was found to be an important factor when evaluating Fe_{NO} values, as seen for other lung function parameters [27, 28, 31, 32].

Lung Function Effect. Although the influence of lung function has been described in few studies [22, 25], it was a significant predictor for Fe_{NO} in the present study. In fact, for the included females, MEF_{50} (%) and TGV (L) explained a slight but significant Fe_{NO} variability. This result is in in agreement with Liu et al. published norms [22], where FVC was included in the reference equation. These authors [22] have extensively described the relationship between lung function and Fe_{NO}.

Why Does Pulmonary Function Influence Fe_{NO} [22]? It has been demonstrated that Fe_{NO} levels may vary with the airway caliber [1], perhaps because of a mechanical effect on NO output. The percent values of MEF_{50}, sensitive to the small airway, are more likely to present underlying lung disease. The absolute value of TGV, indicating lung size more rationally than a percentage of predicted values, is sensitive to lung hyperinflation and so for small airways [27]. Given that MEF_{50} correlated with Fe_{NO} only in females, one wonders whether females' small airways produced more NO. The hypothesis of flow dependence advanced by Liu et al. [22] cannot be retained, since FVC and FEV_1 were not positively associated with Fe_{NO} values.

Conversely, sex, age, weight, BMI, BSA, and obesity status were not significantly associated with Fe_{NO} when other variables were controlled. In the published studies [9–24] the following significant influencing factors were found: race, sex, age, weight, BMI, household smoke exposure, and session exam.

4.5. Why Are not the Findings about the Fe_{NO} Determinants Consistent with Previous Literature? Many explanations can be advanced especially about methodological factors and inclusion of additional significant influencing factors.

Methodological Factors. The low percentage variance explained by the retained reference equation ($r^2 = 2\%$) reveals the possible difficulty to determine the effect of different exogenous factors and their combination with Fe_{NO} [7]. For example, the effect of atopy cannot easily be captured in a single factor, because atopy may result in an increase in Fe_{NO} of anywhere between zero and several hundred ppb depending on the degree of IgE sensitization and the level of allergen exposure. However, this does not rule out the benefit of adjusting for the more predictive effect of, for example, age, height, and sex on expected normal Fe_{NO} values. Another source of variation of the reference equations published [9–24] may be the use of different Fe_{NO} analysers or calibration procedures [50] or the method of measure (Medisoft versus NIOX) [45], even though all studies reported that they were following the ATS/ERS guidelines [1, 51]. As Fe_{NO} values obtained with different devices are not directly comparable and may differ to a clinically relevant, as the device is used [44], the present data were adjusted according to Brooks et al. [45]. As can be seen (Figure 1) and even after adjustment (Supplemental Figure 3), the present study mean ± SD measured Fe_{NO} was significantly overestimated by the Saudi Arabian male reference equations [16]. Sample sizes, age groups, race-ethnic constituencies, and noninclusion criteria of reference populations in other studies [9–24] make it difficult to compare findings. Therefore, care must be taken when comparing the present study Fe_{NO} results with those using different machines in different studies [9–24]. Thus, the use of other studies Fe_{NO} norms may lead to misinterpretation of the Fe_{NO} values. The definition and future use of specific guidelines on how to report studies on reference values may contribute to the standardization of reports [7]. Published Fe_{NO} recommendations [1] are helpful in the standardization of the measurement, but not in the standardization, of how the methods are described [7].

Inclusion of Additional Significant Influencing Factors. Additional significant influencing factors were included in adults' Fe_{NO} norms [9–24]: race, ethnicity, atopy, allergy, total IgE, serum eosinophil cationic protein, smoking status, interaction between sex and smoking habits, asthma diagnosis, ambient NO, and upper respiratory tract infection symptoms. In addition, interindividual differences in NO synthase basal levels (e.g., variants in the neuronal NOS 1 gene [20, 21, 52]) can account for the missing variability. The effects of race and atopy are analyzed in the following sections and the other additional influencing factors are discussed in the Supplemental Data.

Race Effect. Among the published studies [9–24], some have included non-Caucasian subjects such as African Americans [15], Arab [16], or Asian [17, 19–24]. The effect of race on Fe_{NO} values is now well established [21, 24] and current data are vastly adequate to allow conclusions about people of other genetic background [7, 21, 24]. There is evidence that race and ethnicity play an important role in lung function prediction [53].

Atopy Effect. For the definition of reference values for Fe_{NO}, atopy is an essential variable and its assessment using questionnaire data is insufficient. There is good evidence that Fe_{NO} mainly reflects atopy in population studies [1, 54]. For that reason, the atopic status of study subjects was determined (using questionnaires) and only healthy nonatopic adults were included.

Jacinto et al. [7] have suggested that the methodology and reporting on normal Fe_{NO} values and the corresponding reference equations should be standardized and the formulation of reference equations should be based on a preset physiological model with endogenous and stable (at least in the short term) factors such as sex, age, and height. Furthermore, the influence of exogenous factors should be minimized in the population under study, for example, by using objective allergy testing and objective markers of exposure to cigarette smoke [7].

4.6. Fe_{NO} Norms and Interpretation. Among the published Fe_{NO} norms for adults [9–24], none have proposed a clear method of interpreting the measured Fe_{NO} or has provided a prospective verification of their studied populations.

Fe_{NO} values can be difficult to interpret, as they are strongly influenced by several intraindividual factors, including anthropometric data, sex, atopy, and smoking habits [7]. This is one of many problems with diagnostic tests, as recently discussed [48]: it is difficult to define "normality" in a given assessment [55]. Moreover, the numeric value of a diagnostic test can be presented in several forms: the absolute value and the percent predicted of a reference value.

The ATS guidelines [2] suggested that decision cut points rather than reference values be used when interpreting Fe_{NO} levels. Specifically, the guidelines stated that an adult $Fe_{NO} < 25$ ppb indicates a low likelihood of eosinophilic inflammation and corticosteroid response, whereas an adult $Fe_{NO} > 50$ ppb indicates otherwise. However, these cut points have not been validated in the Arab Tunisian population. At the heart of determining cut points is the definition of "normality," which can be taken as representing 95% of the healthy general population [9, 21]. Based on this assumption, the present study showed that values exceeding 26 ppb for adults 18 to 60 years of age indicated abnormality and a high risk of airway inflammation. Coincidentally, the ATS threshold of 25 ppb for adults is very close to the maximum value observed in the Arab Tunisian population. The ATS upper threshold of 50 ppb for 18 to 60 years of age was 24 ppb above the maximum value and could possibly be lowered as proposed by See and Christiani [21].

Due to the inadequacy of the Saudi Arabian males' Fe_{NO} reference equations [16], norms adapted to Arab Tunisian population were established. For practical and routine interpretation of Fe_{NO}, two ways were proposed: normal absolute values range taking into consideration age and height ranges and a reference equation taking height into account.

The interpretation of Fe_{NO} currently involves the use of absolute values reported in ppb, both in clinical practice and research, although absolute values are seldom used in respiratory medicine diagnostic tests [7]. According to the present study, we recommend the use of the total sample Fe_{NO} maximum value as a threshold, and each adult Fe_{NO} value higher than 26 ppb will be considered as abnormal. This method is much simpler for clinicians to remember and device manufacturers to program. In practice, it has been proposed that a "personal best" value for Fe_{NO} might be used [7, 56]. This is a strong approach if the objective is to monitor Fe_{NO}. However, for the initial assessment of Fe_{NO} in a patient, this method is questionable [7]. Furthermore, the personal best values were shown to be close to published reference values [7, 56].

The percentage predicted of the reference value is now a standard transformation in most lung function laboratories [48]. Thus, their use to calculate reference values may be a practical and clinically useful approach [7]. Jacinto et al. [7] suggested the use of a similar approach when interpreting Fe_{NO} values using the percentage predicted of the reference value. A reference equation should include only easily measured anthropometric data that appear to influence Fe_{NO}. For a practical interest, and as sex does not significantly affect the Fe_{NO} value, authors recommend the use of the total sample reference equation (Box 2) when calculating a predicted Fe_{NO} value. As recommended [40], the ULN to add to the predicted value was mentioned. The observed Fe_{NO} for each individual is then deemed to be abnormally high if it exceeds the ULN of the predicted mean [21]. This would allow clinicians to individualize decision making according to the unique characteristics of each person. To the best of our knowledge, the present study is the first to suggest a clear way to interpret a measured Fe_{NO} value. However, Leon de la Barra et al. [8] stated that correcting Fe_{NO} using reference equations did not enhance the performance characteristics of Fe_{NO} as a predictor of either the diagnosis of asthma or steroid responsiveness in patients with chronic airways related symptoms.

Further research is needed to clarify the Fe_{NO} method of interpretation [7]. Nevertheless, the individual factors taken into consideration will be an important step to improve the interpretation of Fe_{NO} values [7]. Such factors are easily accessible at the clinic and incorporating them will require very little extra effort [7]. Most importantly, if reference equations are used, clinical cut-offs can be generalized across age groups and genetic backgrounds [7].

4.7. Reliability of the Local Fe_{NO} Norms. The reliability of the retained norms was confirmed in the prospectively studied population, confirming the continuing need of establishing regional reference norms [1]. This argues for the use of specific reference norms in the Arab Tunisian population. The implications of this for adults with bronchial asthma may be considerable, resulting in a false-positive misdiagnosis of bronchial inflammation.

In conclusion, reliable norms to interpret the results of Fe_{NO} were established in healthy Tunisian Arab adults. The Fe_{NO} can easily be predicted according to a reference equation taking into consideration height or age and height table ranges. Local Fe_{NO} norms enrich the World Bank of Fe_{NO} norms the clinician can use to choose the most appropriate norms based on an adult's location or ethnic group.

Abbreviation List

ATS: American Thoracic Society
BMI: Body mass index
BSA: Body surface area
ERS: European Respiratory Society
Fe_{NO}: Fraction of nitric oxide in exhaled breath
FEV1: 1st second forced expiratory volume
FVC: Forced vital capacity
IgE: Immunoglobulin E
LLN: Lower limit of normal
ln: Natural logarithm
MEFx%: Forced expiratory flow when x% of FVC has been exhaled
MMEF: Maximal mid expiratory flow
PEF: Peak expiratory flow
ppb: Parts per billion
r: Correlation coefficient
r^2: Determination coefficient
RSD: Residual standard deviation
RV: Residual volume
SD: Standard deviation
TGV: Thoracic gas volume
TLC: Total lung capacity
ULN: Upper limit of normal
95% CI: 95% confidence interval.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

Authors wish to thank Professor Béchir SAADAOUI for his invaluable contribution in the improvement of the quality of the writing in the present paper.

References

[1] S. American Thoracic and S. European Respiratory, "ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide," *The American Journal of Respiratory and Critical Care Medicine*, vol. 171, pp. 912–930, 2005.

[2] R. A. Dweik, P. B. Boggs, S. C. Erzurum et al., "An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (Fe_{NO}) for clinical applications," *The American Journal of Respiratory and Critical Care Medicine*, vol. 184, no. 5, pp. 602–615, 2011.

[3] P. J. Barnes, R. A. Dweik, A. F. Gelb et al., "Exhaled nitric oxide in pulmonary diseases a comprehensive review," *Chest*, vol. 138, no. 3, pp. 682–692, 2010.

[4] F. L. M. Ricciardolo, "Multiple roles of nitric oxide in the airways," *Thorax*, vol. 58, no. 2, pp. 175–182, 2003.

[5] A. D. Smith, J. O. Cowan, S. Filsell et al., "Diagnosing asthma: comparisons between exhaled nitric oxide measurements and conventional tests," *The American Journal of Respiratory and Critical Care Medicine*, vol. 169, no. 4, pp. 473–478, 2004.

[6] I. D. Pavord and N. Martin, "Will exhaled nitric oxide monitoring become routine in managing asthma?" *Expert Review of Respiratory Medicine*, vol. 3, no. 2, pp. 107–111, 2009.

[7] T. Jacinto, K. Alving, R. Correia, A. Costa-Pereira, and J. Fonseca, "Setting reference values for exhaled nitric oxide: a systematic review," *Clinical Respiratory Journal*, vol. 7, no. 2, pp. 113–120, 2013.

[8] S. Leon de la Barra, A. D. Smith, J. O. Cowan, G. Peter Herbison, and D. Robin Taylor, "Predicted versus absolute values in the application of exhaled nitric oxide measurements," *Respiratory Medicine*, vol. 105, no. 11, pp. 1629–1634, 2011.

[9] M. Olivieri, G. Talamini, M. Corradi et al., "Reference values for exhaled nitric oxide (reveno) study," *Respiratory Research*, vol. 7, article 94, 2006.

[10] P. Maestrelli, S. Ferrazzoni, A. Visentin et al., "Measurement of exhaled nitric oxide in healthy adults," *Sarcoidosis Vasculitis and Diffuse Lung Diseases*, vol. 24, no. 1, pp. 65–69, 2007.

[11] A. Olin, B. Bake, and K. Torén, "Fraction of exhaled nitric oxide at 50 mL/s: reference values for adult lifelong never-smokers," *Chest*, vol. 131, no. 6, pp. 1852–1856, 2007.

[12] D. R. Taylor, P. Mandhane, J. M. Greene et al., "Factors affecting exhaled nitric oxide measurements: the effect of sex," *Respiratory Research*, vol. 8, article 82, 2007.

[13] J. Travers, S. Marsh, S. Aldington et al., "Reference ranges for exhaled nitric oxide derived from a random community survey of adults," *The American Journal of Respiratory and Critical Care Medicine*, vol. 176, no. 3, pp. 238–242, 2007.

[14] H. Dressel, D. de la Motte, J. Reichert et al., "Exhaled nitric oxide: independent effects of atopy, smoking, respiratory tract infection, gender and height," *Respiratory Medicine*, vol. 102, no. 7, pp. 962–969, 2008.

[15] M. C. Levesque, D. W. Hauswirth, S. Mervin-Blake et al., "Determinants of exhaled nitric oxide levels in healthy, non-smoking African American adults," *Journal of Allergy and Clinical Immunology*, vol. 121, no. 2, pp. 396.e3–402.e3, 2008.

[16] S. S. Habib, A. A. Abba, M. A. Al-Zoghaibi, and M. M. Subhan, "Reference range values of fractional exhaled nitric oxide in healthy Arab adult males," *Saudi Medical Journal*, vol. 30, no. 11, pp. 1395–1400, 2009.

[17] K. Matsunaga, T. Hirano, T. Kawayama et al., "Reference ranges for exhaled nitric oxide fraction in healthy Japanese adult population," *Allergology International*, vol. 59, no. 4, pp. 363–367, 2010.

[18] A. F. Gelb, S. C. George, F. Camacho, C. Fraser, C. F. Taylor, and S. Shakkottai, "Increased nitric oxide concentrations in the small airway of older normal subjects," *Chest*, vol. 139, no. 2, pp. 368–375, 2011.

[19] Y. Zhang and J. Lin, "Normal values and influencing factors of fractional exhaled nitric oxide for Chinese healthy adults," *National Medical Journal of China*, vol. 92, no. 28, pp. 1971–1973, 2012.

[20] F. W. Ko, T. F. Leung, G. W. Wong et al., "Determinants of, and reference equation for, exhaled nitric oxide in the Chinese population," *European Respiratory Journal*, vol. 42, pp. 767–775, 2013.

[21] K. C. See and D. C. Christiani, "Normal values and thresholds for the clinical interpretation of exhaled nitric oxide levels in the US general population: results from the National Health and Nutrition Examination Survey 2007–2010," *Chest*, vol. 143, no. 1, pp. 107–116, 2013.

[22] H. Liu, J. Hsu, Y. Cheng, and M. Chou, "Exhaled nitric oxide in a Taiwanese population: age and lung function as predicting factors," *Journal of the Formosan Medical Association*, vol. 108, no. 10, pp. 772–777, 2009.

[23] S. Kim, T. H. Kim, J. W. Sohn, H. J. Yoon, D. H. Shin, and S. S. Park, "Reference values and determinants of exhaled nitric oxide in healthy korean adults," *Journal of Asthma*, vol. 47, no. 5, pp. 563–567, 2010.

[24] D. J. Brody, X. Zhang, B. K. Kit et al., "Reference values and factors associated with exhaled nitric oxide: U.S. youth and adults," *Respiratory Medicine*, vol. 107, pp. 1682–1691, 2013.

[25] S. Rouatbi, A. Alqodwa, S. Ben Mdella, and H. Ben Saad, "Fraction of exhaled nitric oxide (Fe_{NO}) norms in healthy North African children 5–16 years old," *Pediatric Pulmonology*, vol. 48, pp. 981–995, 2013.

[26] H. Ben Saad, M. Tfifha, I. Harrabl et al., "Factors influencing pulmonary function in Tunisian women aged 45 years and more," *Revue des Maladies Respiratoires*, vol. 23, no. 4, pp. 324–338, 2006.

[27] M. Bougrida, H. Ben Saad, M. Kheireddinne Bourahli, I. Bougmiza, and H. Mehdioui, "Spirometric reference equations for Algerians aged 19 to 73 years," *Revue des Maladies Respiratoires*, vol. 25, no. 5, pp. 577–590, 2008.

[28] H. Ben Saad, S. Rouatbi, S. Raoudha et al., "Vital capacity and peak expiratory flow rates in a north-African population aged 60 years and over: influence of anthropometric data and parity," *Revue des Maladies Respiratoires*, vol. 20, no. 4, pp. 521–530, 2003.

[29] H. Ben Saad, M. N. El Attar, K. Hadj Mabrouk et al., "The recent multi-ethnic global lung initiative 2012 (GLI2012) reference values don't reflect contemporary adult's North African spirometry," *Respiratory Medicine*, vol. 107, pp. 2000–2008, 2013.

[30] H. Ben Saad, C. Prefaut, Z. Tabka et al., "6-minute walk distance in healthy North Africans older than 40 years: influence of parity," *Respiratory Medicine*, vol. 103, no. 1, pp. 74–84, 2009.

[31] S. Rouatbi, H. Ben Saad, I. Latiri, Z. Tabka, and H. Guénard, "North-african reference values of alveolar membrane diffusion capacity and pulmonary capillary blood volume," *Respiration*, vol. 80, no. 4, pp. 301–312, 2010.

[32] S. Rouatbi, Y. F. Ouahchi, C. Ben Salah et al., "Physiological factors influencing pulmonary capillary volume and membrane diffusion," *Revue des Maladies Respiratoires*, vol. 23, no. 3, pp. 211–218, 2006.

[33] H. Ben Saad, H. Selmi, K. Hadj Mabrouk et al., "Spirometric "Lung Age" estimation for North African population," *Egyptian Journal of Chest Disease and Tuberculosis*, vol. 63, pp. 491–503, 2014.

[34] K. Suresh and S. Chandrashekara, "Sample size estimation and power analysis for clinical research studies," *Journal of Human Reproductive Sciences*, vol. 5, no. 1, pp. 7–13, 2012.

[35] H. Ben Saad, "The narghile and its effects on health, part I: the narghile, general description and properties," *Revue de Pneumologie Clinique*, vol. 65, no. 6, pp. 369–375, 2009.

[36] H. Ben Saad, "The narghile and its effects on health, part II: the effects of the narghile on health," *Revue de Pneumologie Clinique*, vol. 66, no. 2, pp. 132–144, 2010.

[37] B. G. Ferris, "Epidemiology standardization project (American Thoracic Society)," *American Review of Respiratory Disease*, vol. 118, no. 6, pp. 1–120, 1978.

[38] World Health Organization, "Obesity: preventing and managing the global epidemic," Report of a WHO Consultation on Obesity, World Health Organization, Geneva, Switzerland, 1998.

[39] D. Dubois and E. Dubois, "A formula to estimate the appropriate surface area if height and weight be known," *Archives of Internal Medicine*, vol. 17, pp. 863–871, 1916.

[40] R. Pellegrino, G. Viegi, V. Brusasco et al., "Interpretative strategies for lung function tests," *European Respiratory Journal*, vol. 26, no. 5, pp. 948–968, 2005.

[41] L. Lehtimäki, H. Kankaanranta, S. Saarelainen, V. Turjanmaa, and E. Moilanen, "Inhaled fluticasone decreases bronchial but not alveolar nitric oxide output in asthma," *European Respiratory Journal*, vol. 18, no. 4, pp. 635–639, 2001.

[42] Z. Tabka, H. Hassayoune, H. Guenard et al., "Spirometric reference values in a Tunisian population," *La Tunisie Médicale*, vol. 73, no. 2, pp. 125–131, 1995.

[43] M. Jenicek and R. Cleroux, "Clinical epidemiology: its evolution and uses in clinical practice and medical research," *Union Medicale du Canada*, vol. 114, no. 8, pp. 625–632, 651, 1985.

[44] S. Korn, I. Telke, O. Kornmann, and R. Buhl, "Measurement of exhaled nitric oxide: comparison of different analysers," *Respirology*, vol. 15, no. 8, pp. 1203–1208, 2010.

[45] C. R. Brooks, S. M. Brogan, C. J. van Dalen, P. K. Lampshire, J. Crane, and J. Douwes, "Measurement of exhaled nitric oxide in a general population sample: a comparison of the Medisoft HypAir Fe_{NO} and Aerocrine NIOX analyzers," *Journal of Asthma*, vol. 48, no. 4, pp. 324–328, 2011.

[46] H. Ghannem and A. Hadj Fredj, "Prevalence of cardiovascular risk factors in the urban population of Soussa in Tunisia," *Journal of Public Health Medicine*, vol. 19, no. 4, pp. 392–396, 1997.

[47] H. Ben Saad, L. Ben Amor, S. Ben Mdalla et al., "The importance of lung volumes in the investigation of heavy smokers," *Revue des Maladies Respiratoires*, vol. 31, pp. 29–40, 2014.

[48] S. Stanojevic, A. Wade, and J. Stocks, "Reference values for lung function: past, present and future," *European Respiratory Journal*, vol. 36, no. 1, pp. 12–19, 2010.

[49] C. Pedroletti, M. Högman, P. Meriläinen, L. S. Nordvall, G. Hedlin, and K. Alving, "Nitric oxide airway diffusing capacity and mucosal concentration in asthmatic schoolchildren," *Pediatric Research*, vol. 54, no. 4, pp. 496–501, 2003.

[50] K. Alving, C. Janson, and L. Nordvall, "Performance of a new hand-held device for exhaled nitric oxide measurement in adults and children," *Respiratory Research*, vol. 7, article 67, 2006.

[51] P. E. Silkoff, "Recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide in adults and children-1999," *The American Journal of Respiratory and Critical Care Medicine*, vol. 160, no. 6, pp. 2104–2117, 1999.

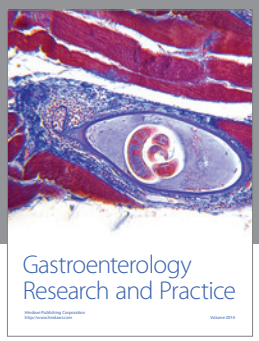
[52] H. Grasemann, K. S. Van's Gravesande, R. Büscher, J. M. Drazen, and F. Ratjen, "Effects of sex and of gene variants in constitutive nitric oxide synthases on exhaled nitric oxide," *The American Journal of Respiratory and Critical Care Medicine*, vol. 167, no. 8, pp. 1113–1116, 2003.

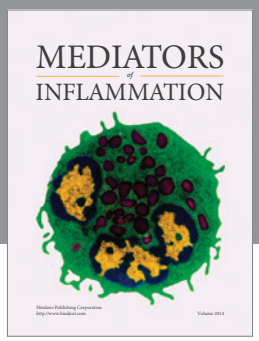
[53] R. Kumar, M. A. Seibold, M. C. Aldrich et al., "Genetic ancestry in lung-function predictions," *The New England Journal of Medicine*, vol. 363, no. 4, pp. 321–330, 2010.

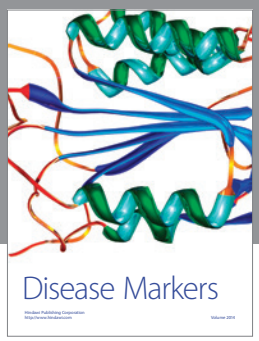
[54] L. Philipp, B. Julia, and G. Matthias, "Exhaled nitric oxide in healthy children: variability and a lack of correlation with atopy," *Pediatric Allergy and Immunology*, vol. 13, no. 1, pp. 37–46, 2002.

[55] D. L. Sackett and R. B. Haynes, "Evidence base of clinical diagnosis: the architecture of diagnostic research," *British Medical Journal*, vol. 324, no. 7336, pp. 539–541, 2002.

[56] A. D. Smith, J. O. Cowan, and D. R. Taylor, "Exhaled nitric oxide levels in asthma: personal best versus reference values," *Journal of Allergy and Clinical Immunology*, vol. 124, no. 4, pp. 714.e4–718.e4, 2009.

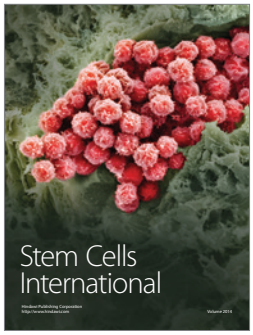


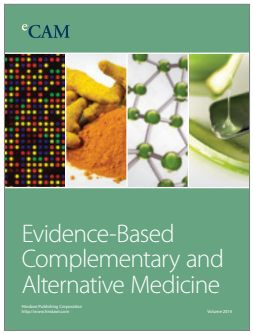


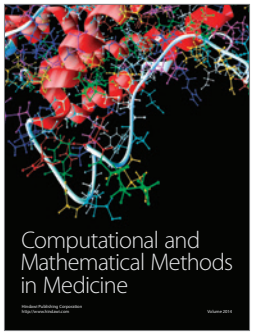


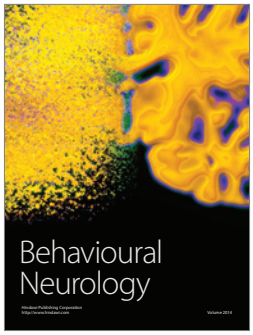
Hindawi

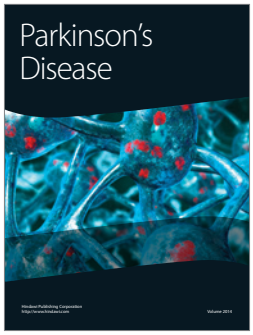
Submit your manuscripts at
http://www.hindawi.com

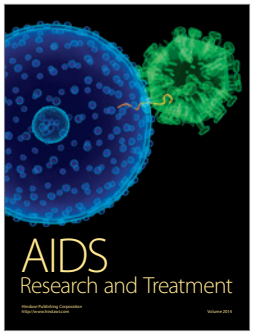


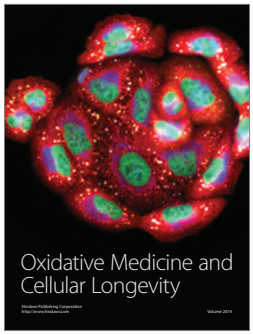












SUPPLEMENTARY DATA

Title. Fraction of Exhaled Nitric Oxide (Fe_{NO}) Norms in Healthy Tunisian Adults

Authors. Sonia ROUATBI, Mohamed Ali CHOUCHENE, Ines SFAXI, Mohamed BEN REJEB, Zouhair TABKA, Helmi BEN SAAD.

POPULATION AND METHODS

Study design

Subjects were recruited from the Hospital workers, the parents of Medical school students and the public. Informational letters clarifying the aims of the study were put up in the Hospital departments and the school of Medicine. When an adult was interested, an appointment for a medical questionnaire and exploration was fixed. Data from each volunteer adult included: sex, age, height, weight, smoking history (active or passive), medication use, medical history, physical examination, Fe_{NO} and plethysmography data. All adults received a copy of their exploration, and when an unknown dysfunction was discovered, they were sent to a specialist.

Statistical analysis

Published *Fe_{NO} norms for Saudi Arabian males*[1] is extensively detailed in the ***Supplementary Table I***.

The model of the natural logarithm linear regression equation is the following: $LnFe_{NO}$ (ppb) = B_1 x categorical $variable_1$ + B_2 x categorical $variable_2$ + B_n x categorical $variable_n$ + Bx_1 x continuous $variable_1$ + Bx_2 x continuous $variable_2$ + Bx_n x continuous $variable_n$ + Constant. B is the non-standardized regression coefficient. 95% CI around each B was determined.

RESULTS

Non-inclusion criteria

Non-inclusion criteria are presented in the **Supplemantary Table II**.

From an initial sample of 400 voluntary adults, non-inclusion criteria were found in 93 subjects. The remaining 307 adults were divided into two groups: **equation** (145 females and 112 males) and **validation** (25 females and 25 males) **groups**'.

Subject's data

Supplementary Table 3 exposes the total sample plethysmographic data expressed in absolute values.

DISCUSSION

Subject's data

As for almost all the studies aiming to publish Fe_{NO} norms[1-15], the present study was not a random population sample. Some caution should be warranted when interpreting the results of cross-sectional studies in volunteers, because of a possible selection bias and cohort effects[16]. Thus, longitudinal studies analyzed by appropriate statistical models are necessary to correctly describe the functional changes associated with age[17].

The present study sample size (n=257) was higher than in some studies [n=106[10], n=121[1], n=122[4], n=166[12], n=193[2], n=200[15], n=204[3], n=240[9], n=249[8]] but was smaller than in some previous studies [n=895[5,7], n=897[11], n=1093[13], n=1131[6]]. It is important to note that the subgroup aged > 55 years with a height

> 1.75 m includes only two subjects and the data obtained in this subgroup cannot be extrapoled to the general population.

The validation group number (n=50) seems small, but it is sufficient to provide adequate validation since it is closer to that included in similar studies aiming to validate Tunisian reference equations for children Fe_{NO} data and adults 6-min walk distance data, respectively, (n=24[18] and n=30[19]).

Inclusion of additional significant influencing factors

Additional significantly influencing factors were included in the published adults' Fe_{NO} norms[1-15,20]: race, ethnicity, atopy, allergy, total IgE, serum eosinophil cationic protein, smoking status, interaction between sex and smoking habits, asthma diagnosis, ambient NO and upper respiratory tract infection symptoms.

Like most of the published studies[1-6,8-10], smoking habits were not assessed with objective methods. For the definition of reference values for Fe_{NO}, smoking status is an essential variable and its assessment using only questionnaire data seems insufficient. Chronically reduced levels of Fe_{NO} have been demonstrated in cigarette smokers in addition to acute effects immediately after cigarette smoking[21,22]. Despite the depressing effect of smoking, smokers with asthma still have a raised Fe_{NO}[23]. For that reason, the ATS/ERS recommend that subjects should not smoke in the hour before measurements, and short- and long-term active and passive smoking history should be recorded[21].

In addition to individual-specific factors, several behavioural and environmental factors have been pointed out as influencing Fe_{NO}[24], such as

rhinovirus infections[7], allergen exposure[25], physical exercise[26], ozone exposure[27], air pollution[28] and nutritional history[16].

What do "abnormal" Fe_{NO} values reflect?

The use of Fe_{NO} in clinical practice has been the focus of numerous studies and considerable debate[29,30]. Vijverberg et al.[30] concluded that High Fe_{NO} is a poor marker of asthma control in children with reported use of asthma medication. Leon de la Barra et al.[29] stated that "there is an emerging consensus, poised somewhere between outright enthusiasm and sceptical nihilism, that the utility of Fe_{NO} is greatest in identifying the potential (or not) for steroid responsiveness and in assessing complex asthma in which there is significant discordance between the symptoms which the patient is reporting, and the intensity of underlying airway inflammation". These two aspects were simultaneously considered by Perez de Llano et al.[31]. The following question was asked: can Fe_{NO} be used to predict patients with poorly controlled asthma, in whom additional inhaled or oral steroid would or would not be helpful? Interestingly, almost 50% did not gain control despite maximum doses of inhaled fluticasone and the addition of oral steroid[31]. Fe_{NO} values of less than 30 ppb were associated with predictive values of >90% for the absence of response to high dose therapy. These results demonstrate the usefulness of Fe_{NO} in directing the clinician away from inappropriate treatment.

Some studies have demonstrated that Fe_{NO} measurement might be useful for the diagnosis of asthma in adults[32,33]. However, in some obese asthmatic patients, very low Fe_{NO} levels were observed[34]. In fact it has been shown in asthma patients that BMI and plasma ratio of leptin/adiponectin is

associated with reduced Fe_{NO} and that BMI is associated with increased exhaled 8-isoprostanes[34]. In addition, Fe_{NO} measurement has also been investigated in the treatment algorithm for asthma. However, randomised controlled algorithm asthma control trials revealed equivocal benefits when adding Fe_{NO} measurement to the routine guideline management including spirometry[35,36].

SUPPLEMENTARY FIGURES LEGENDS

Supplementary Figure 1. Distribution of the Tunisian healthy total sample by sex and age range.

***p < 0.05:** females vs. males.

Supplementary Figure 2. The measured fraction-of-exhaled-nitric oxide (Fe_{NO}) in subgroups of 257 healthy Tunisian adults', according to age (**FigA**), height (**FigB**) and weight (**FigC**) ranges. n=number of adults. Data are shown as box-and-whiskers-plots illustrating the mean ( ), standard error ( ) and 95% confidence interval ( ).

***p<0.05:** Comparison (T-test) from one range to the next.

NS: not significant.

Supplementary Figure 3. Comparison, for the same age range, of measured and predicted (after adjustment according to Brooks et al.) fraction-of-exhaled-nitric-oxide (Fe_{NO}) determined from Saudi Arabian norms:

Fig 3A. Model including weight.

Fig 3B. Model including body mass index.

n=number of males having the age range of the Saudi Arabian predicted Fe_{NO} study.

Solid line (▬▬▬): regression line. Dashed line (▪▪▪▪▪▪▪): identity line. **r^2:** coefficient of determination. **r:** correlation coefficient. **p**: probability

Supplementary Figure 4. The measured fraction-of-exhaled-nitric-oxide (Fe_{NO}) values of the equation and validation groups according to height.

O: Males of the Equation group (n=112)

●: Males of the Validation group (n=25)

□: Females of the Equation group (n=145)

■ : Females of the Validation group (n=25)

Supplementary Table 1. Fraction-of-exhaled-nitric-oxide (Fe_{NO}) norms for Arab populations.

	Kingdom of Saudi Arabia: Habib et al.[1]	**Tunisia (present study)**
Period of study	September 2007- August 2008	May 2012- December 2012
Time of Fe_{NO} test	9 a.m to 11 a.m	8 a.m to 12 a.m
Recruitment method	Medical students and hospital personnel	Convenience sample: medical students and hospital personnel
Non-inclusion criteria	Smoking, recent/Current upper RTI, medications, atopy, clinical manifestations of allergic diseases, abnormal spirometry, abnormal serum IgE levels, respiratory disease, symptoms of respiratory disease (last 1 year)	Hay fever, chronic illnesses, oto-rhino-laryngolocic diseases or symptoms, clinical manifestation of allergic diseases, pulmonary diseases or related respiratory symptoms, abnormal lung function, pregnancy, regular medication use, current or ex-smokers, inability to perform properly respiratory measurements.
Precautions	Refrain from eating, drinking	Avoid eating (2 h), drinking (2

	and strenuous exercise 2 h before	h), ingesting caffeine (2 h), strenuous activity (2 h)
Guidelines	ATS/ERS$_{2005}$[21]	ATS/ERS$_{2005}$[21]
Material	Chemiluminescence analyzer (NOX EVA 4000, Seres, aix-en-provence, France) *Calibration* before each test	Electrochemical analyzer (Medisoft, Sorinnes [Dinant], Belgium) *Calibration* before each test
Technique	Online	Online
Flow (ml/s)	50±10%	50
Other explorations	Questionnaire, spirometry, total IgE	Questionnaire, plethysmography
Individual Fe_{NO}	Mean of 3 exhalations within 10% deviation	Mean of 3 exhalations within 10% deviation
Race	Arab	Arab
Sex	M (n=121)	M (n=112); F (n=145)
Age (Yr)	31.00±12.24* 19-64**	39±12* 38 to 41***
Height (m)	1.72±0.08* 1.49-1.88**	1.65±0.09* 1.64 to 1.66***
Fe_{NO} (ppb)	22.8±8.1* 7.7-46.6**	13.54±4.87* 12.94 to 14.14***
Fe_{NO} influencing factors	W, BMI	H

Fe_{NO} reference equation	**Model 1.** Fe_{NO}=47.096 - 0.119xW (kg) **Model 2.** Fe_{NO}=31.541-0.289xBMI (kg/m^2).	**Male:** $LnFe_{NO}$ (ppb) = 4.470-1.111xH (m). **Female:** $LnFe_{NO}$ (ppb) = 4.734-1.170xH (m)-0.002xMEF_{50} (%)-0.061xTGV (L) **Total sample:** $LnFe_{NO}$ (ppb) = 3.467-0.561xH (m) Any Fe_{NO} value greater than 26.00 ppb may be considered abnormal.
r^2 and RSD	NR	**M:** r^2=3.80%. RSD=0.390. **F:** r^2=9.24%. RSD=0.332. **TS:** r^2=1.92%. RSD=0.365.
Interpretation method	84% of subjects: Fe_{NO} < 30 ppb 95% of subjects: Fe_{NO} < 40 ppb	Upper limit of normal
Validation group	No	Yes
Comparison groups	No	No
Comparison with other	No	Yes

studies

ATS: American thoracic society. **BMI:** body mass index. **ERS:** European respiratory society. **F:** female. **H:** height. **h:** hour. **IgE:** immunoglobulin E. **Ln:** natural logarithm. **M:** male. **MEF_x:** forced expiratory flow when x% of FVC has been exhaled. **n:** number. **NR:** not reported. **r^2:** coefficient determination. **RSD:** residual standard deviation. **RTI**: respiratory tract infection. **TGV:** thoracic gas volume. **TS:** total sample. **W:** weight

:** data are mean±SD. *:** data are minimum-maximum. *******: data are 95% confidence interval.

1

2

Supplementary Table 2. Applied non-inclusion criteria (n=400)

Brief description	Number
Recent airway infection (cold, flu, sore throat within the last 7 days)	**21**
Current or ex-smokers	**18**
Poor cooperation to achieve a standard spirometry or a correct fraction-of-exhaled-nitric-oxide measure	**15**
Atopic history (allergic rhinitis, urticaria, recurrent symptoms of rhinitis, skin allergy)	**14**
Asthma or obstructive ventilatory defect	**12**
Medication use	**7**
Eating or/and drinking (water, caffeine) before measurement	**6**

Supplementary Table 3. Healthy Arab Tunisian never-smoking adults' plethysmographic data expressed in absolute values.

		Females (n=145)	Males (n=112)	Total sample (n=257)
FVC	(L)	3.14±0.51	4.30±0.69*	3.65±0.83
FEV_1	(L)	2.63±0.47	3.47±0.60*	2.99±0.67
FEV_1/FVC	(absolute value)	0.83±0.06	0.81±0.06*	0.82±0.06
PEF	(L/s)	5.08±1.02	7.11±1.27*	5.97±1.52
MMEF	(L/s)	2.93±0.86	3.53±1.00*	3.19±0.97
MEF_{25}	(L/s)	1.39±0.66	1.57±0.84	1.47±0.75)
MEF_{50}	(L/s)	3.52±0.92	4.30±1.16*	3.86±1.10
MEF_{75}	(L/s)	4.77±0.99	6.64±1.26*	5.58±1.45
TLC	(L)	4.53±0.64	6.16±1.05*	5.24±1.17
TGV	(L)	2.56±0.56	3.48±0.98*	2.96±0.89
RV	(L)	1.50±0.48	1.96±0.76*	1.70±0.66

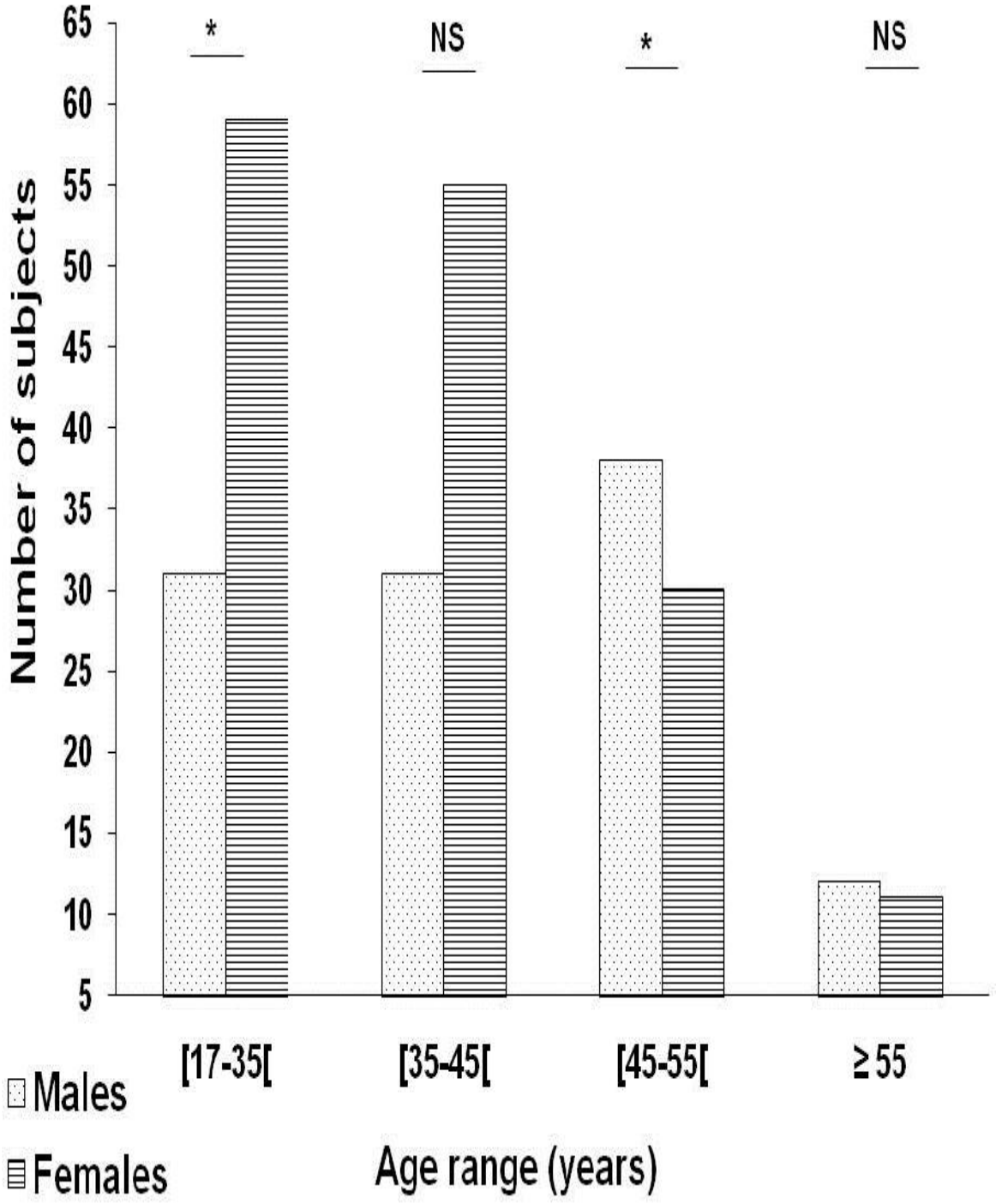
For abbreviations, see abbreviations list. *$p < 0.05$ (Mann-Whitney U test): females vs. males.

Supplementary Table 4. Equation and validation groups' data.

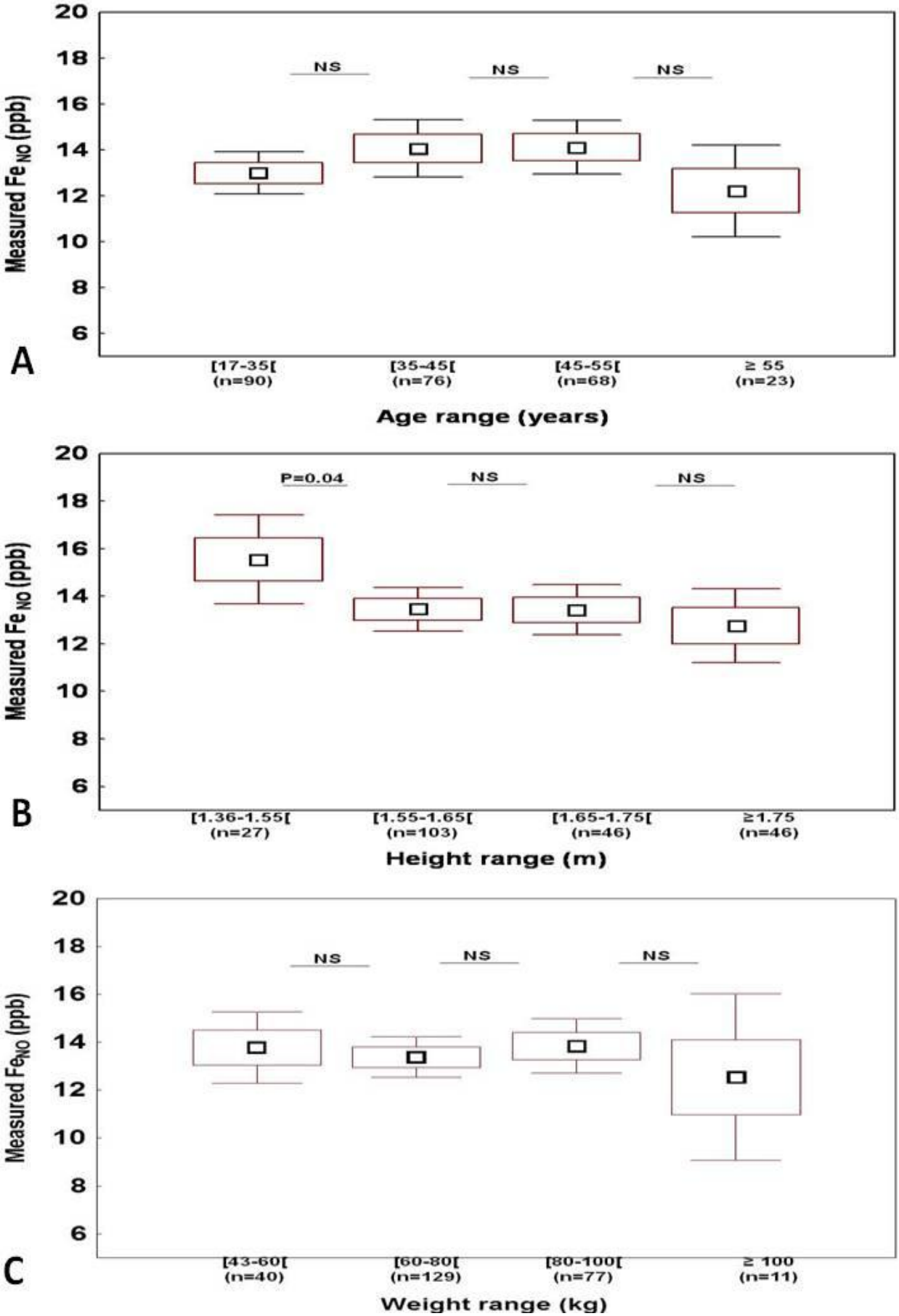
		Equation group (n=257)	Validation group (n=50)
Anthropometric data (data are mean±SD)			
Sex	(Male/female)	112/145	25/25
Age	(year)	39.37±11.58	40.88±13.45
Weight	(kg)	74±14	75±22
Height	(m)	1.65±0.09	1.66±0.09
Body mass index	($kg.m^{-2}$)	27±5	28±9
Body surface area	(m^2)	1.81±0.19	1.82±0.24
Plethysmographic and fraction-of-exhaled-nitric-oxide (Fe_{NO}) data (data are mean±SD))			
FVC	(%)	97±12	93±14*
FEV_1	(%)	94±11	91±12*
PEF	(%)	79±14	79±14
MMEF	(%)	86±20	83±22
MEF_{25}	(%)	74±31	77±39
MEF_{50}	(%)	86±20	81±17
MEF_{75}	(%)	84±16	83±17
TLC	(%)	94±12	93±14
TGV	(%)	101±24	105±32
RV	(%)	101±34	116±44*
Fe_{NO}	(ppb)	13.54±4.87	12.98±4.83
Obesity status (data are number (%))			
Obesity status	Normal weight	95 (37%)	18 (36%)
	Overweight	96 (37%)	15 (30%)
	Obesity	66 (26%)	17 (34%)

For abbreviations, see abbreviations list. Plethysmographic data are expressed as a percentage predicted value (%).
*$p < 0.05$ (Mann-Whitney U test): Equation group vs. Validation group.
**$p < 0.05$ (chi-2): Equation group vs. Validation group.

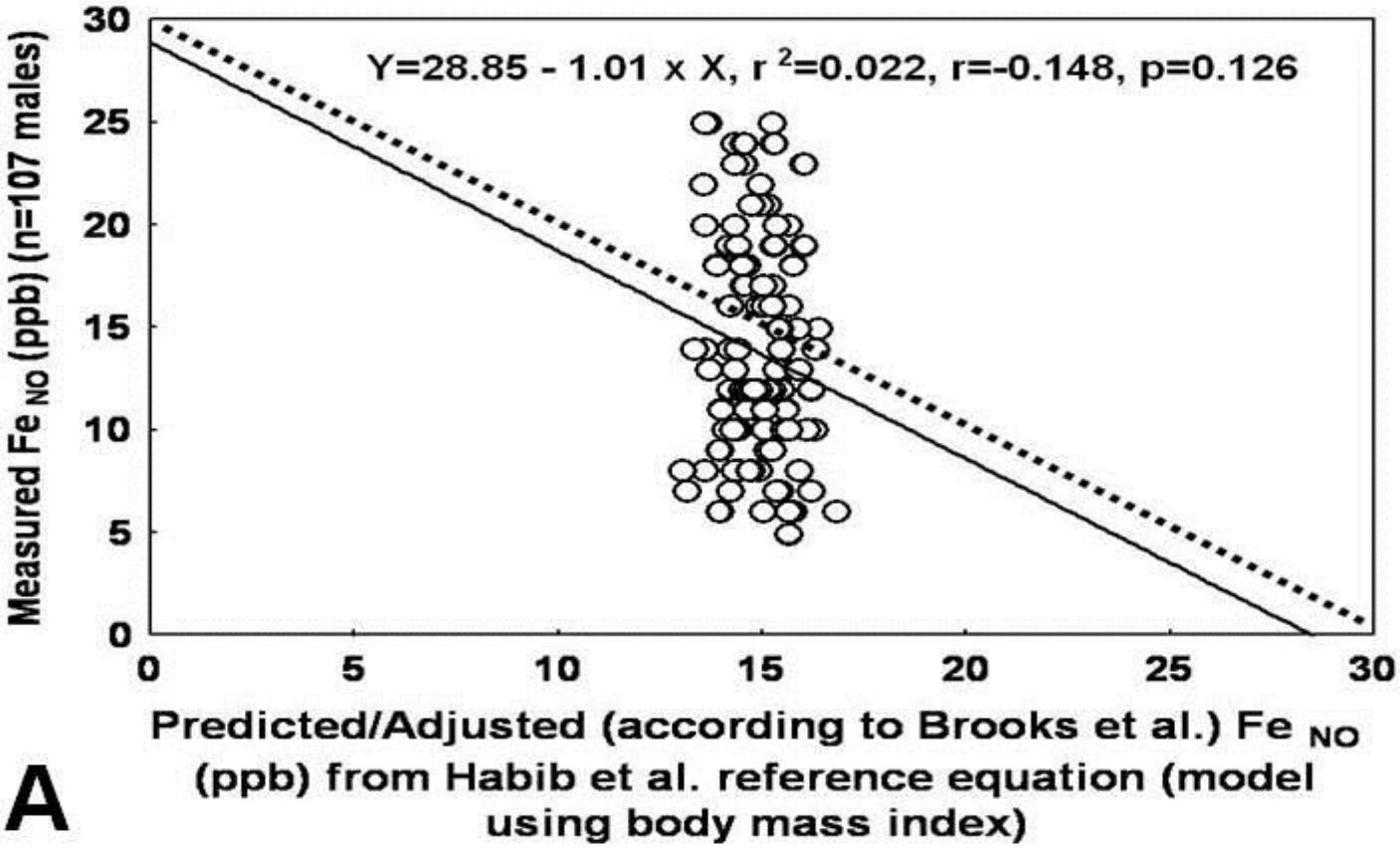
Supplementary Figure 1.



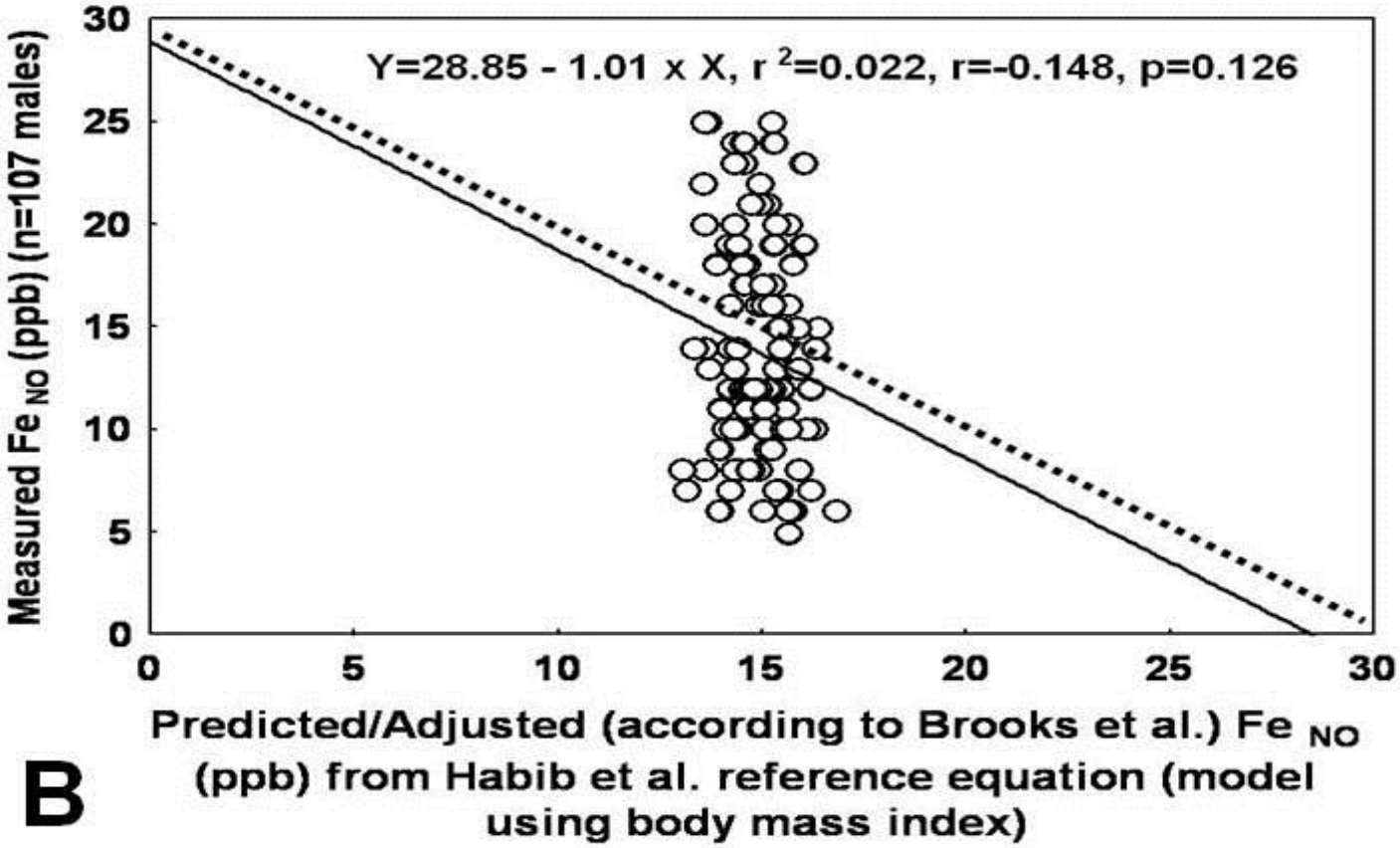
Supplementary Figure 2.



Supplementary Figure 3.

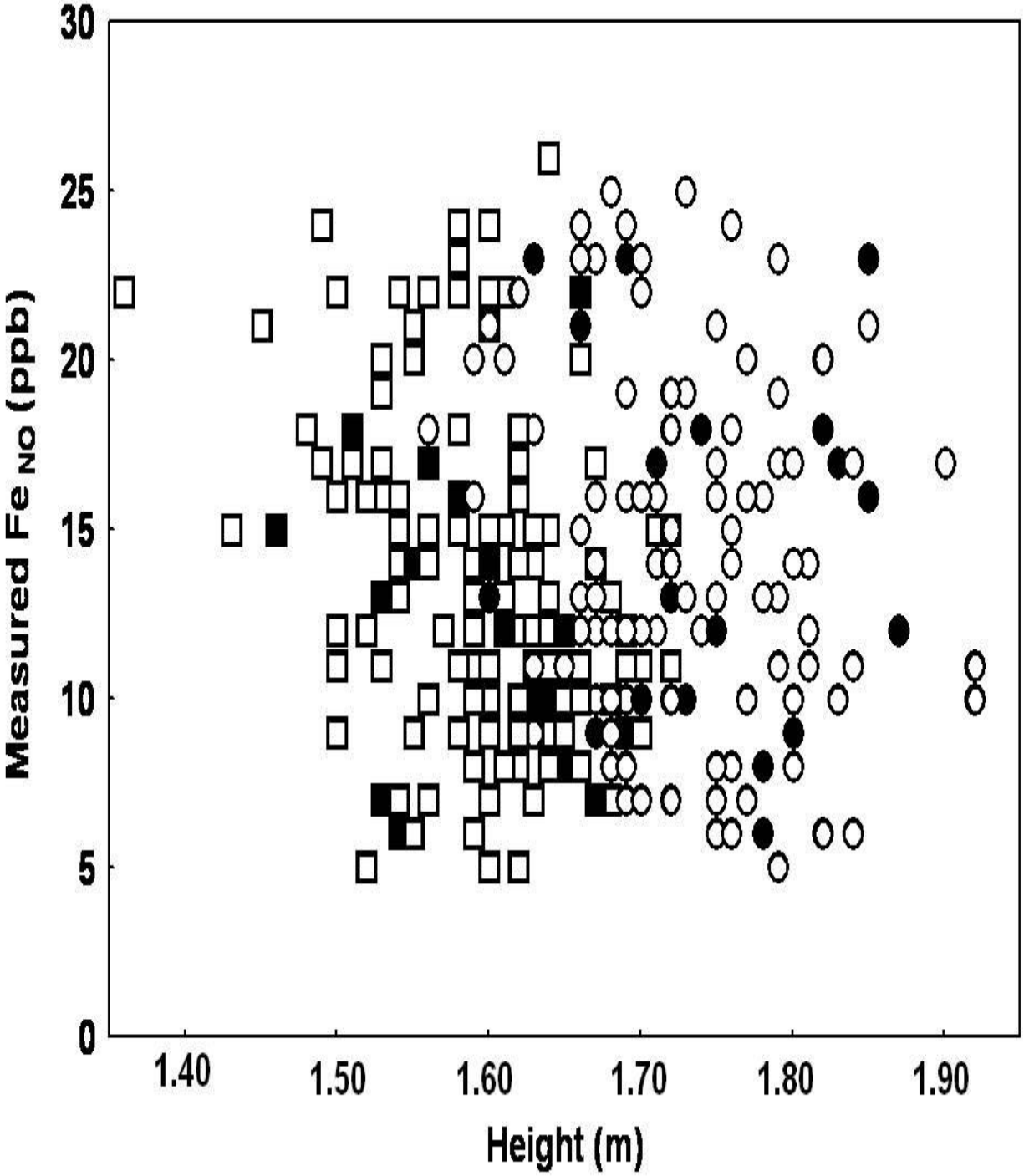


A



B

Supplementary Figure 4.



REFERENCES

1 Habib SS, Abba AA, Al-Zoghaibi MA, et al. Reference range values of fractional exhaled nitric oxide in healthy Arab adult males. Saudi Med J 2009; 30:1395-1400

2 Travers J, Marsh S, Aldington S, et al. Reference ranges for exhaled nitric oxide derived from a random community survey of adults. Am J Respir Crit Care Med 2007; 176:238-242

3 Olivieri M, Talamini G, Corradi M, et al. Reference values for exhaled nitric oxide (reveno) study. Respir Res 2006; 7:94

4 Maestrelli P, Ferrazzoni S, Visentin A, et al. Measurement of exhaled nitric oxide in healthy adults. Sarcoidosis Vasc Diffuse Lung Dis 2007; 24:65-69

5 Taylor DR, Mandhane P, Greene JM, et al. Factors affecting exhaled nitric oxide measurements: the effect of sex. Respir Res 2007; 8:82

6 Olin AC, Bake B, Toren K. Fraction of exhaled nitric oxide at 50 mL/s: reference values for adult lifelong never-smokers. Chest 2007; 131:1852-1856

7 Levesque MC, Hauswirth DW, Mervin-Blake S, et al. Determinants of exhaled nitric oxide levels in healthy, nonsmoking African American adults. J Allergy Clin Immunol 2008; 121:396-402 e393

8 Liu HC, Hsu JY, Cheng YW, et al. Exhaled nitric oxide in a Taiwanese population: age and lung function as predicting factors. J Formos Med Assoc 2009; 108:772-777

9 Matsunaga K, Hirano T, Kawayama T, et al. Reference ranges for exhaled nitric oxide fraction in healthy Japanese adult population. Allergol Int 2010; 59:363-367

10 Gelb AF, George SC, Camacho F, et al. Increased nitric oxide concentrations in the small airway of older normal subjects. Chest 2011; 139:368-375

11 Dressel H, de la Motte D, Reichert J, et al. Exhaled nitric oxide: independent effects of atopy, smoking, respiratory tract infection, gender and height. Respir Med 2008; 102:962-969

12 Kim SH, Kim TH, Sohn JW, et al. Reference values and determinants of exhaled nitric oxide in healthy Korean adults. J Asthma 2010; 47:563-567

13 Ko FW, Leung TF, Wong GW, et al. Determinants of, and reference equation for, exhaled nitric oxide in the Chinese population. Eur Respir J 2013; 42:767-775

14 See KC, Christiani DC. Normal values and thresholds for the clinical interpretation of exhaled nitric oxide levels in the US general population: results from the National Health and Nutrition Examination Survey 2007-2010. Chest 2013; 143:107-116

15 Zhang YM, Lin JT. [Normal values and influencing factors of fractional exhaled nitric oxide for Chinese healthy adults]. Zhonghua Yi Xue Za Zhi 2012; 92:1971-1973

16 Brooks CR, Brogan SB, van Dalen CJ, et al. Measurement of exhaled nitric oxide in a general population sample: a comparison of the Medisoft HypAir FE(NO) and Aerocrine NIOX analyzers. J Asthma 2011; 48:324-328

17 Pellegrino R, Viegi G, Brusasco V, et al. Interpretative strategies for lung function tests. Eur Respir J 2005; 26:948-968

18 Rouatbi S, Alqodwa A, Ben Mdella S, et al. Fraction of exhaled nitric oxide (Fe(NO)) norms in healthy North African children 5-16 years old. Pediatr Pulmonol 2012

19 Ben Saad H, Prefaut C, Tabka Z, et al. 6-minute walk distance in healthy North Africans older than 40 years: influence of parity. Respir Med 2009; 103:74-84

20 Brody DJ, Zhang X, Kit BK, et al. Reference values and factors associated with exhaled nitric oxide: U.S. youth and adults. Respir Med 2013; 107:1682-1691

21 American Thoracic S, European Respiratory S. 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 2005; 171:912-930

22 Kharitonov SA, Robbins RA, Yates D, et al. Acute and chronic effects of cigarette smoking on exhaled nitric oxide. Am J Respir Crit Care Med 1995; 152:609-612

23 Persson MG, Zetterstrom O, Agrenius V, et al. Single-breath nitric oxide measurements in asthmatic patients and smokers. Lancet 1994; 343:146-147

24 Jacinto T, Alving K, Correia R, et al. Setting reference values for exhaled nitric oxide: a systematic review. Clin Respir J 2012

25 Bodini A, Peroni D, Loiacono A, et al. Exhaled nitric oxide daily evaluation is effective in monitoring exposure to relevant allergens in asthmatic children. Chest 2007; 132:1520-1525

26 Verges S, Tonini J, Flore P, et al. Exhaled nitric oxide in single and repetitive prolonged exercise. J Sports Sci 2006; 24:1157-1163

27 Olin AC, Stenfors N, Toren K, et al. Nitric oxide (NO) in exhaled air after experimental ozone exposure in humans. Respir Med 2001; 95:491-495

28 Delfino RJ, Staimer N, Gillen D, et al. Personal and ambient air pollution is associated with increased exhaled nitric oxide in children with asthma. Environ Health Perspect 2006; 114:1736-1743

29 Leon de la Barra S, Smith AD, Cowan JO, et al. Predicted versus absolute values in the application of exhaled nitric oxide measurements. Respir Med 2011; 105:1629-1634

30 Vijverberg SJ, Koster ES, Koenderman L, et al. Exhaled NO is a poor marker of asthma control in children with a reported use of asthma medication: a pharmacy-based study. Pediatr Allergy Immunol 2012; 23:529-536

31 Perez-de-Llano LA, Carballada F, Castro Anon O, et al. Exhaled nitric oxide predicts control in patients with difficult-to-treat asthma. Eur Respir J 2010; 35:1221-1227

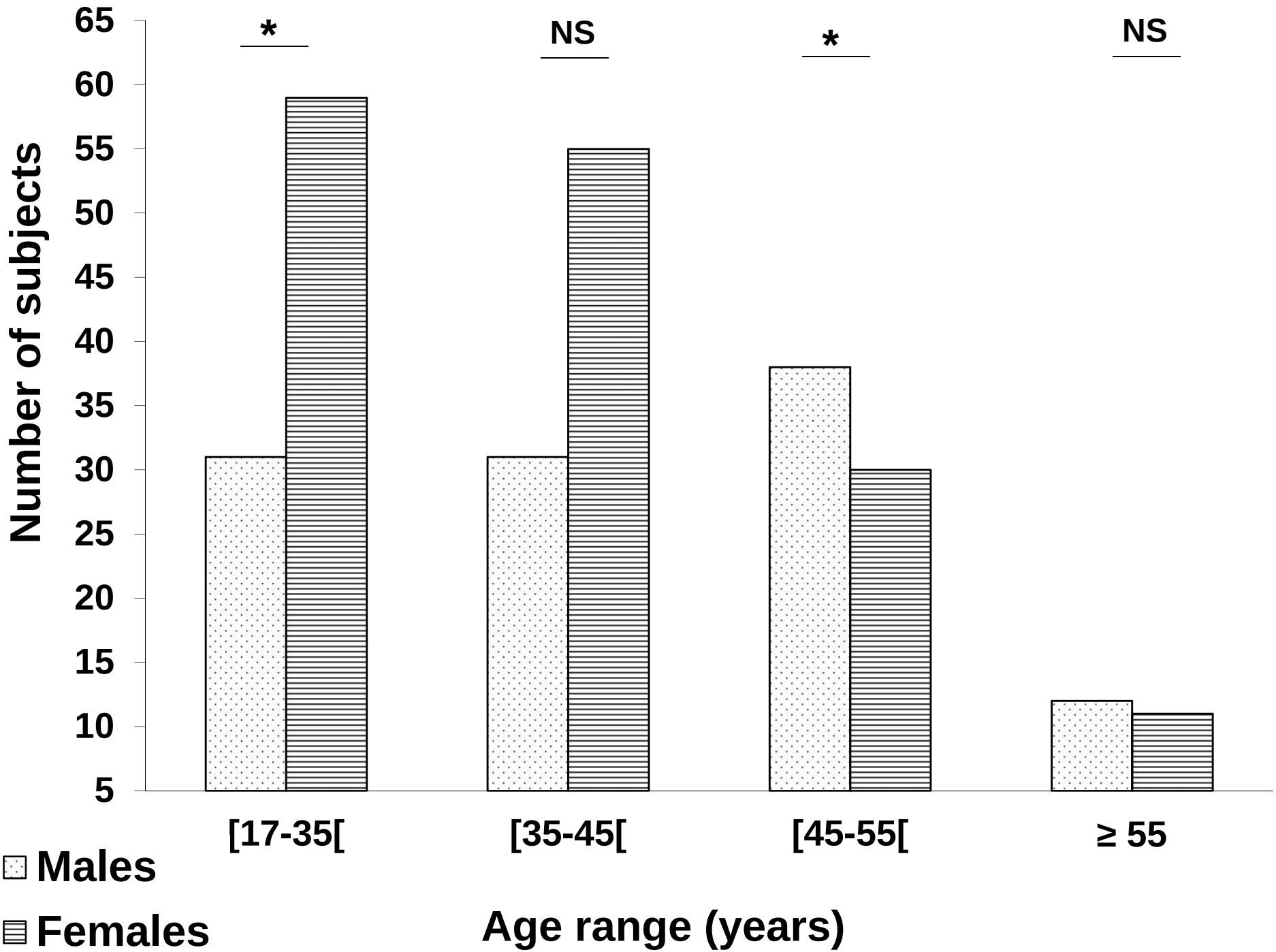
32 Smith AD, Cowan JO, Filsell S, et al. Diagnosing asthma: comparisons between exhaled nitric oxide measurements and conventional tests. Am J Respir Crit Care Med 2004; 169:473-478

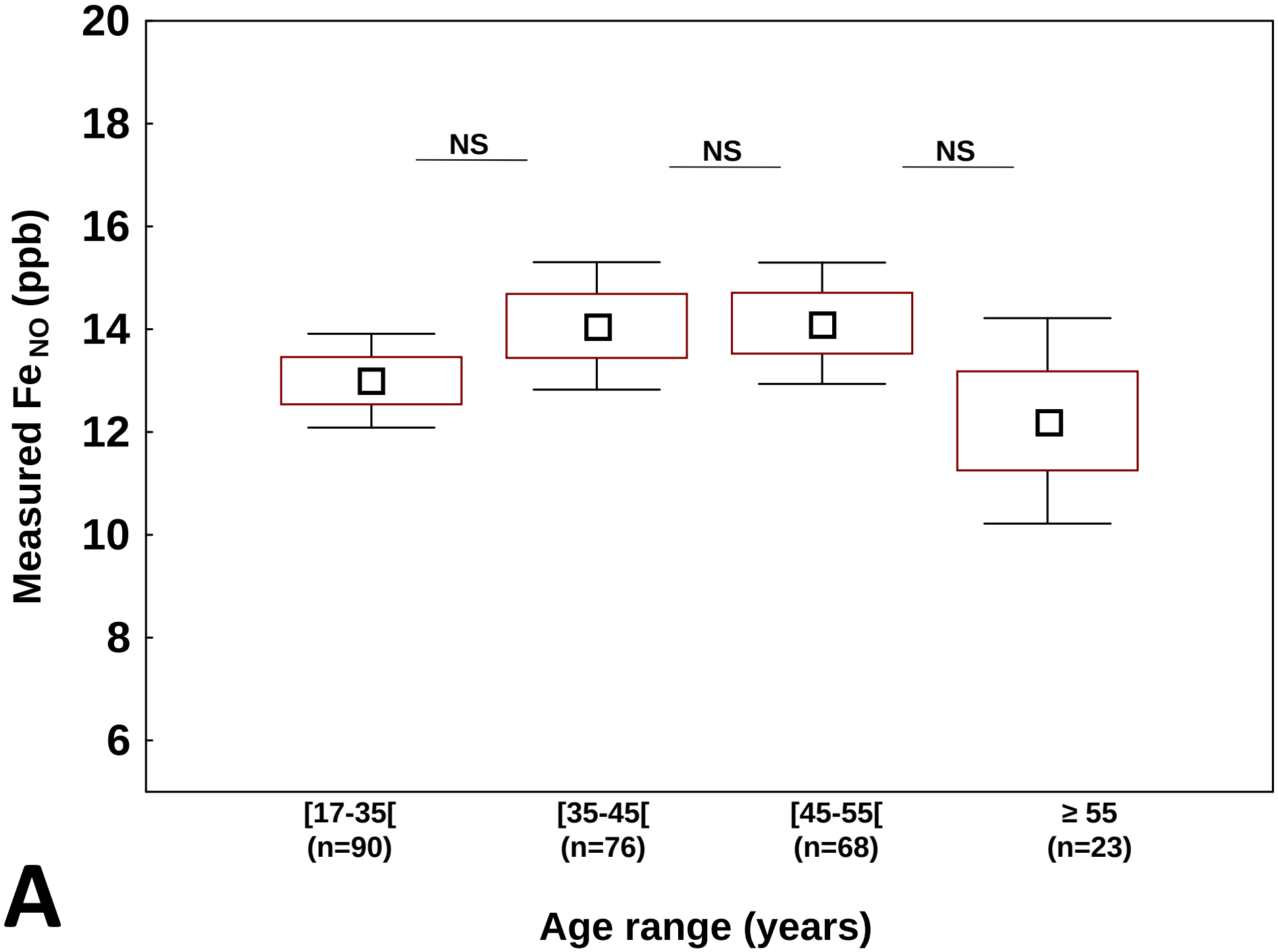
33 Dupont LJ, Demedts MG, Verleden GM. Prospective evaluation of the validity of exhaled nitric oxide for the diagnosis of asthma. Chest 2003; 123:751-756

34 Komakula S, Khatri S, Mermis J, et al. Body mass index is associated with reduced exhaled nitric oxide and higher exhaled 8-isoprostanes in asthmatics. Respir Res 2007; 8:32

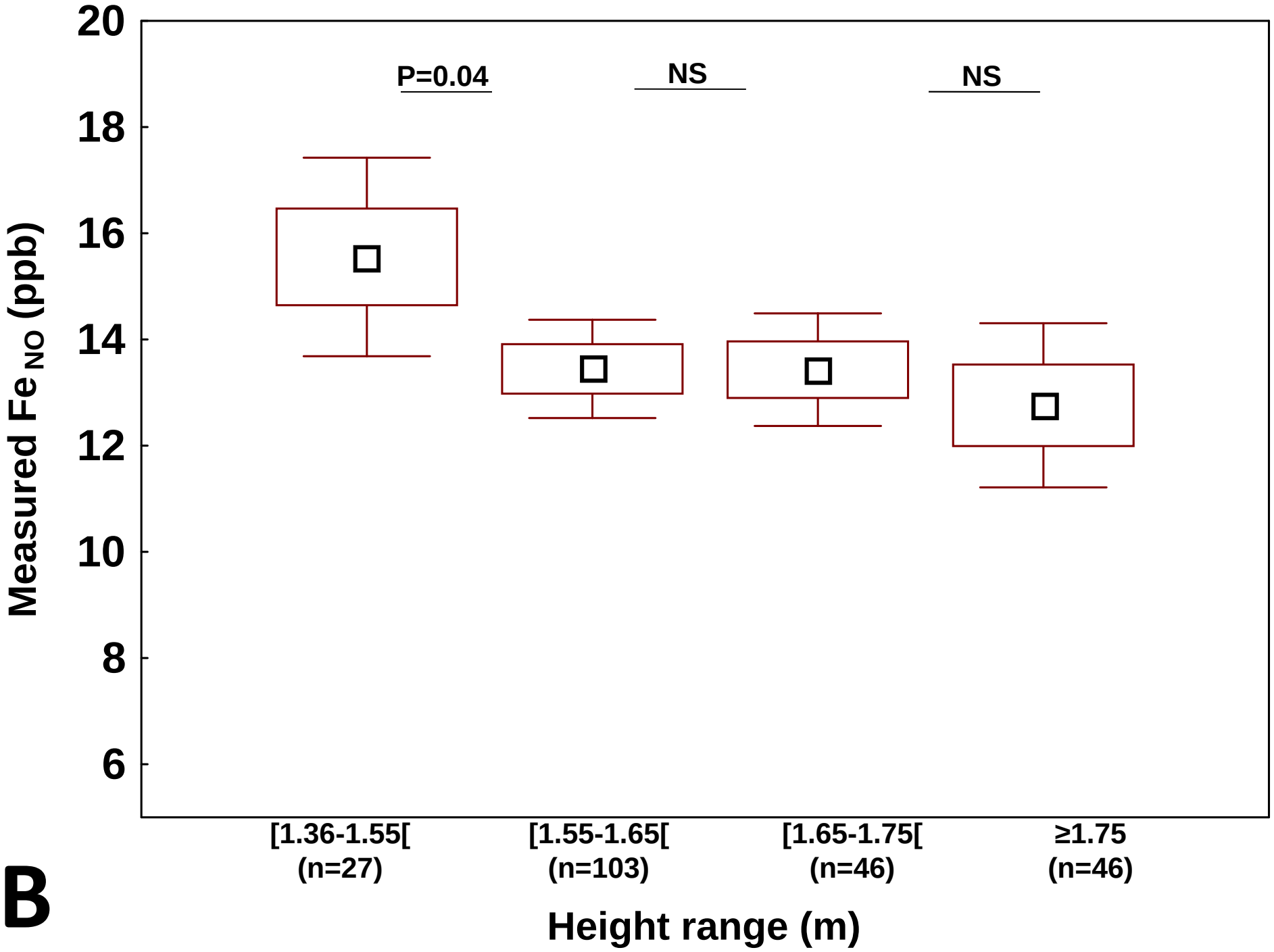
35 Barnes PJ, Dweik RA, Gelb AF, et al. Exhaled nitric oxide in pulmonary diseases: a comprehensive review. Chest 2010; 138:682-692

36 Petsky HL, Cates CJ, Li A, et al. Tailored interventions based on exhaled nitric oxide versus clinical symptoms for asthma in children and adults. Cochrane Database Syst Rev 2009:CD006340

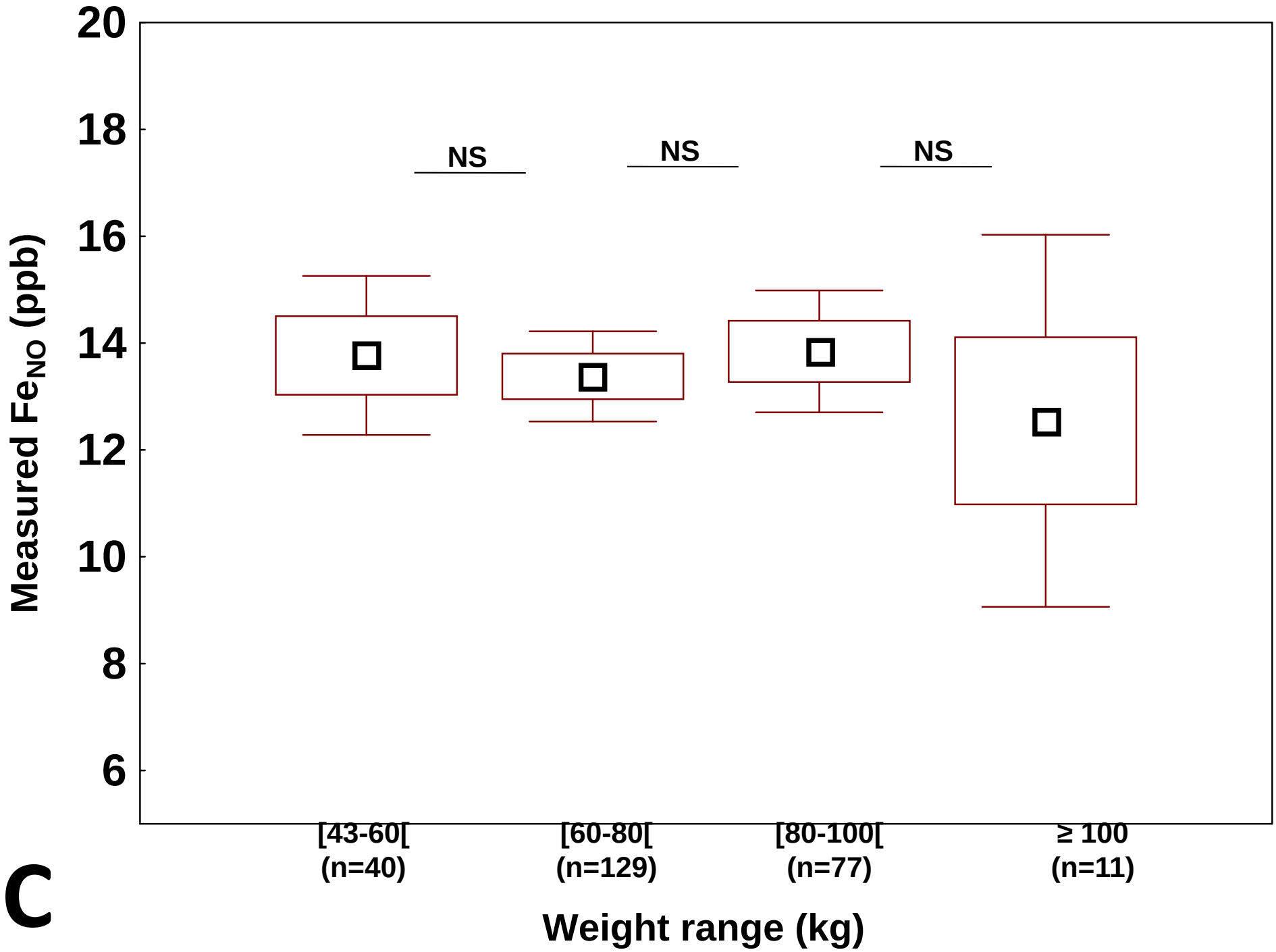

65
60
55
50
45
40
35
30
25
20
15
10
5
Number of subjects
*
NS
*
NS
[17-35[
[35-45[
[45-55[
≥ 55
Males
Females
Age range (years)


20
18
16
14
12
10
8
6
Measured Fe_{NO} (ppb)
NS
NS
NS
[17-35[
(n=90)
[35-45[
(n=76)
[45-55[
(n=68)
≥ 55
(n=23)
Age range (years)

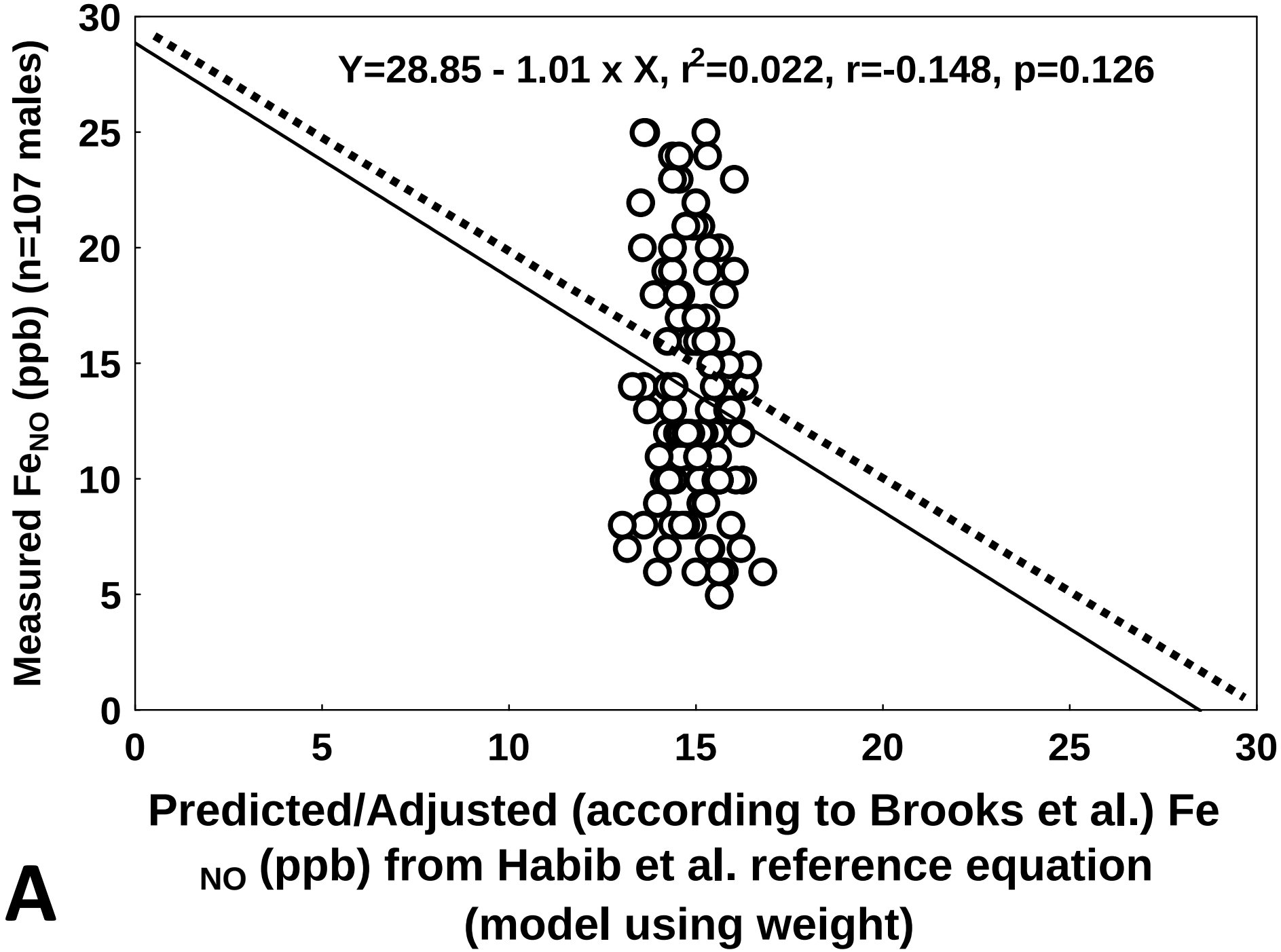
A


20
18
16
14
12
10
8
6
Measured Fe_{NO} (ppb)
P=0.04
NS
NS
[1.36-1.55[
(n=27)
[1.55-1.65[
(n=103)
[1.65-1.75[
(n=46)
≥1.75
(n=46)
Height range (m)

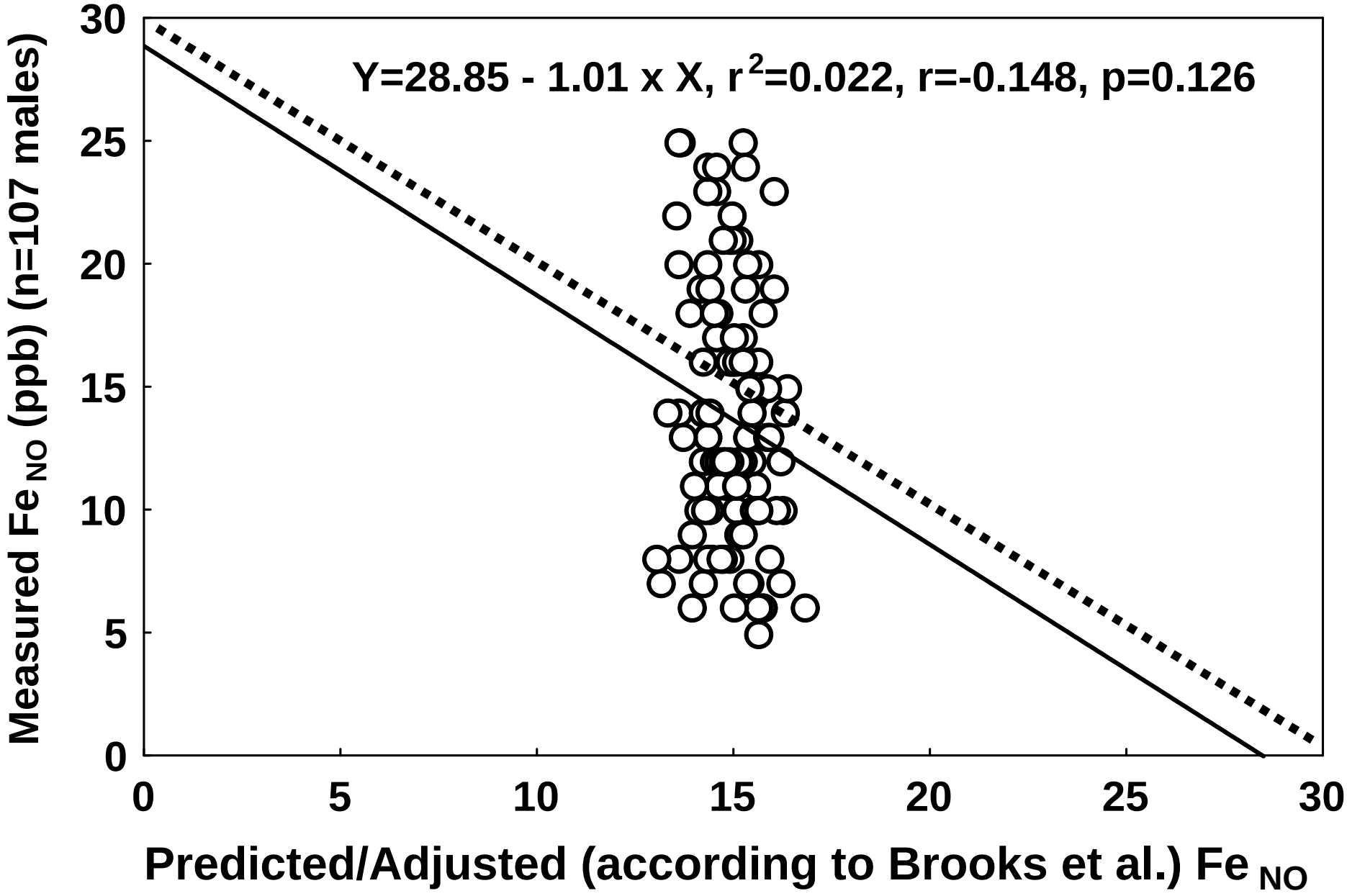
B


20
18
16
14
12
10
8
6
Measured Fe_{NO} (ppb)
NS
NS
NS
[43-60[
(n=40)
[60-80[
(n=129)
[80-100[
(n=77)
≥ 100
(n=11)
Weight range (kg)

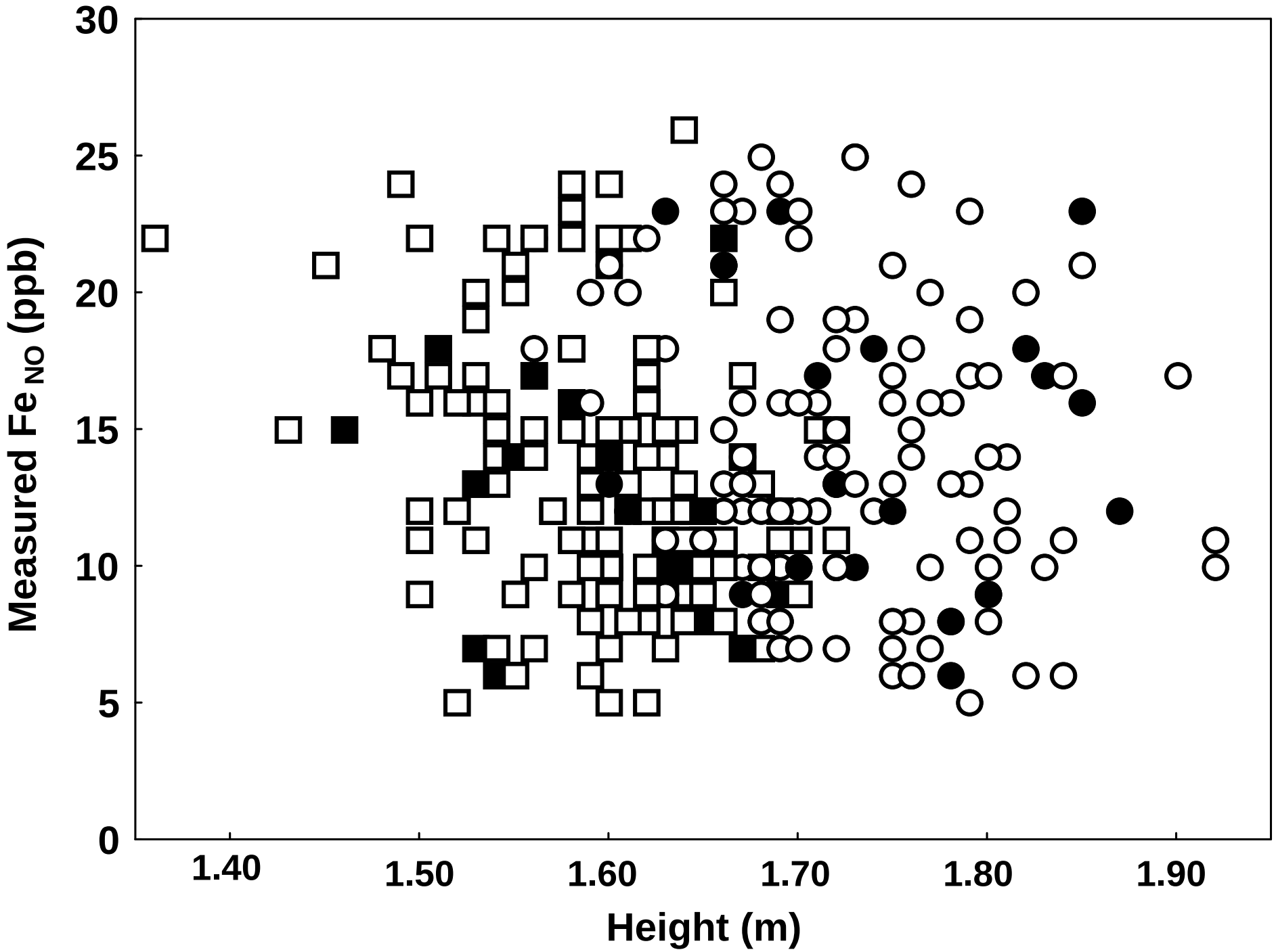
C


Y=28.85 - 1.01 x X, r2=0.022, r=-0.148, p=0.126
Measured FeNO (ppb) (n=107 males)
Predicted/Adjusted (according to Brooks et al.) FeNO (ppb) from Habib et al. reference equation (model using weight)
0
5
10
15
20
25
30

A


Y=28.85 - 1.01 x X, r2=0.022, r=-0.148, p=0.126
Measured FeNO (ppb) (n=107 males)
Predicted/Adjusted (according to Brooks et al.) FeNO (ppb) from Habib et al. reference equation (model using body mass index)
0
5
10
15
20
25
30

B


30
25
20
15
10
5
0
Measured Fe NO (ppb)
1.40
1.50
1.60
1.70
1.80
1.90
Height (m)