The Clinical Respiratory Journal REVIEW ARTICLE

Setting reference values for exhaled nitric oxide: a systematic review

Tiago Jacinto[1,2,3], Kjell Alving[4], Ricardo Correia[1,2], Altamiro Costa-Pereira[1,2] and João Fonseca[1,2,3,5]

1 CINTESIS – Center for Research in Health Technologies and Information Systems, Faculty of Medicine, University of Porto, Porto, Portugal
2 Health Information and Decision Sciences, Faculty of Medicine, University of Porto, Porto, Portugal
3 Instituto CUF – Laboratório de Alergia Inflamação e Respiração, Matosinhos, Portugal
4 Department of Women's and Children's Health, Uppsala University, Uppsala, Sweden
5 Allergy Division, Hospital S. João EPE, Porto, Portugal

Abstract

Background: The values obtained when the fraction of exhaled nitric oxide (FeNO) is measured are affected by several factors that are specific to the individual patient, making interpretation difficult, especially in the initial assessment of patients with respiratory symptoms.

Methods: Systematic review of studies on FeNO reference values and individual-specific factors that influence them.

Results: From 3739 references, 15 studies were included. Four studies included children and adolescents. In nine studies, samples were selected from the general population. Most studies reported objective measures for atopy (nine studies), but not for smoking status (one). Significant determinants of FeNO values reported were age and height (seven studies), atopy (six), smoking (four), weight (four), sex (three) and race (three). Additional factors were included in eight studies. R^2 was reported in only five studies. The logarithmic transformation of FeNO was inadequately described in seven studies.

Conclusion: There are several equations for FeNO reference values that may be used in clinical practice, although the factors they include and the statistical methods they use vary considerably. We recommend the development of standard methods for the evaluation of normal FeNO data and that reference equations should be formulated based on a predetermined physiological model.

Please cite this paper as: Jacinto T, Alving K, Correia R, Costa-Pereira A and Fonseca J. Setting reference values for exhaled nitric oxide: a systematic review. *Clin Respir J* 2013; 7: 113–120.

Key words
exhaled nitric oxide – reference values – systematic review

Correspondence
Tiago Jacinto, MSc, Biostatistics and Medical Informatics, Faculty of Medicine, University of Porto, 4000Porto, Portugal.
Tel: +351225513622
Fax: +351225513623
email: tiagojacinto@med.up.pt

Received: 16 December 2011
Revision requested: 05 June 2012
Accepted: 20 June 2012

DOI:10.1111/j.1752-699X.2012.00309.x

Authorship and contributorship
TJ, JAF and KA analyzed the data. TJ wrote the manuscript. KA and JAF edited the manuscript. RC and ACP revised the manuscript and gave conceptual advice.All authors discussed the results and implications and commented on the manuscript at all stages.

Conflict of interest
TJ and JF received an unrestricted grant from Aerocrine for the development of a fraction of exhaled nitric oxide interpretation aid tool (http://feno.med.up.pt).
KA is a minority shareholder and associate of Aerocrine AB.
RC and ACP have no conflict of interest to declare.

Introduction

The measurement of the fraction of exhaled nitric oxide (FeNO) is now recognized as an accurate, reproducible and completely non-invasive diagnostic test for airway disease (1).

FeNO correlates significantly with bronchial hyperresponsiveness as measured by indirect or direct methods (2) and with the degree of immunoglobulin E (IgE) sensitization (3–5). Furthermore, FeNO is associated with eosinophilia in patients with asthma, e.g. as measured in sputum (6) and bronchial biopsies (7, 8). FeNO decreases quickly after the start of anti-inflammatory therapy when airway inflammation is suppressed and increases after withdrawal of therapy (9–11).

The measurement of FeNO has been used in many settings, but one of the most studied clinical application is the initial assessment of patients with respiratory symptoms (12). However, FeNO values can be difficult to interpret, as they are strongly influenced by several intra-individual factors, including age, height, weight, sex, atopy and smoking habits (13). This is one of many problems with diagnostic tests, as recently discussed (14): it is difficult to define 'normality' in a given assessment (15, 16). Moreover, the numeric value of a diagnostic test can be presented in several forms: the absolute value, the per cent of predicted of a reference value and z-scores. Absolute values are rarely used in lung function tests; however, they are presently used in the interpretation of FeNO. It has been proposed that a 'personal best' value for FeNO might be used (17). This is a strong approach if the objective is to monitor FeNO. However, for the initial assessment of FeNO in a patient, this method cannot be used. Furthermore, the personal best values were shown to be close to published reference values (17). Thus, the use of equations to calculate reference values may be a practical and clinically useful approach.

The aim of this study is to conduct a systematic review of the published literature on the analysis of reference/normative values and on the effects of individual factors (age, sex, height, weight, smoking status and atopy) on FeNO values in healthy individuals.

Material and methods

Eligibility criteria

The inclusion criteria were (i) study sample includes healthy individuals, with no history of asthma, rhinitis or other respiratory or allergic diseases; (ii) measurement of FeNO should be made using the online method, with a constant exhalation flow rate of 50 ± 5 mL/s, according to international recommendations (18); (iii) factors included are one or more of these: age, gender, height, weight, smoking status or atopy (additional factors in the study analysis were allowed and were described); (iv) NO analyser brand and model is specified; and (v) statistical analysis includes a simple or multivariate linear regression in which FeNO is the or one of the dependent variable(s).

Exclusion criteria are (i) animal or *in vitro* testing; (ii) less than 50 healthy subjects assessed with respect to FeNO values; and (iii) studies about other factors such as exercise, environmental exposures and concomitant non-respiratory, genetic or immunologic diseases.

Search strategy

Studies were identified by searching PubMed, SCOPUS and ISI Web of Knowledge from January 1990 to December 2010. The reference list from published international guidelines for the measurement of exhaled nitric oxide (18) was also included. Our search was limited to English-language articles and included unpublished studies. Abstracts, letters, reviews and editorials without original data were not included.

The final search included the terms: *exhaled nitric oxide*, *feno*, *eno*, *reference values*, *normative values*, *normal values, healthy individuals*, *age*, *gender*, *height*, *weight* (or combined as *BMI*), *smoking status* and *atopy*.

The query used in Medline was: *[(smok* OR cigarette OR tobacco) OR (age OR age factors) OR (weight OR body weight) OR (height OR body height) OR (gender OR sex)] AND (exhaled nitric oxide OR eNO OR FENO) AND (healthy individuals OR (reference values OR normal values OR normative values) OR effect)*. Efforts were made to gather all full-text papers, including contact with authors.

Study selection

Study selection had two phases. The first phase was analysis of the title and abstract of the studies found in the databases search. Two independent reviewers (TJ and JAF) classified all studies as included or not. When the abstract of the study did not provide sufficient information to decide on inclusion or exclusion, the study was allowed to the next phase.

The second phase was the analysis of the full-text paper of the study. Again, two reviewers (TJ and JAF) independently classified the study as included or not. Unweighted Kappa was calculated.

Data extraction

The variables extracted from the included studies were: sampled population and country of origin, number and age of participants on whom measurements of exhaled nitric oxide were performed, smoking habits and atopy assessment methodology and exhaled nitric oxide analyser. In addition, the statistical regression model used in the study, dependent variable(s), intercept values, significant factors and the R^2 value were noted.

Results

We screened 3739 references [1367 in PubMed, 1243 in Scopus, 1029 in ISI and 20 from the American

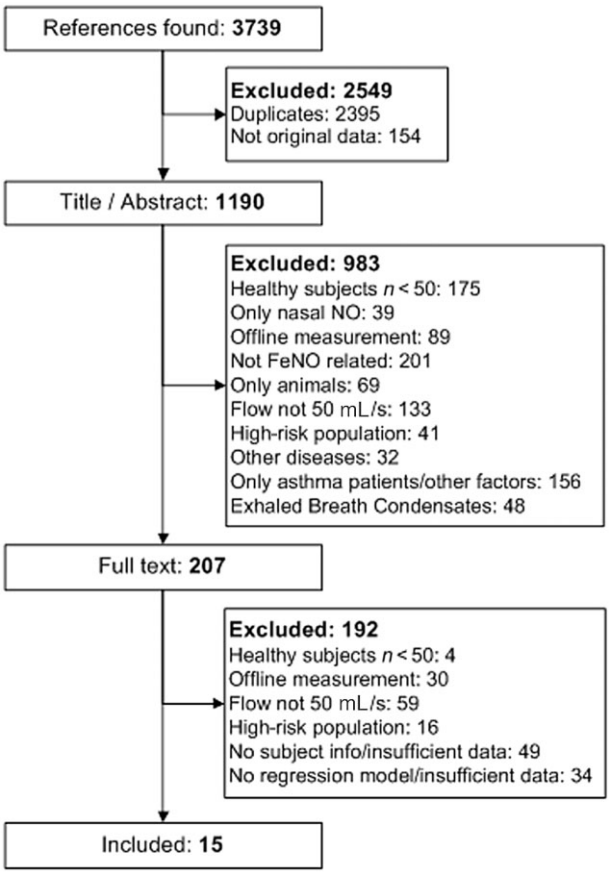

Figure 1. Systematic review flow chart. FENO, fraction of exhaled nitric oxide.

Thoracic Society / European Respiratory Society (ATS/ERS) guidelines] from which were retrieved 207 potentially eligible full articles. Unweighted Kappa was 0.83 [95% confidence interval (CI) 0.74–0.92]. Review flow chart is shown in Fig. 1.

Fifteen studies were included.

The characteristics of the included studies are shown in Table 1.

Four studies (26%) were performed on children and adolescents, and in nine studies (60%), the samples were selected from the general population. The median (min–max) number of participants in each study was 524 (114–2200). Atopy was assessed using skin prick tests in four studies, questionnaires in three, total IgE in three studies and specific IgE in two studies. Only one study reported objective measurement of smoking status (serum cotinine levels) (19). Methods for assessment of atopy and smoking status were not reported in one study (20).

The detailed analysis of the factors and statistical methods of the included studies is shown in Table 2.

In eight studies, the dependent variable (FeNO) was logarithmically transformed: natural logarithm ($n = 3$), decadic logarithm ($n = 1$) and unknown logarithmic base ($n = 7$). The intercept of the models was reported in nine studies. The R^2 value was reported in five studies.

Age and height were significant factors in seven studies, atopy in six, smoking in four, weight in four and sex and race in three studies. Additional factors were included in eight studies. Asthma diagnosis was the most frequent factor ($n = 4$). Other factors were upper respiratory tract infection ($n = 2$), forced vital capacity ($n = 2$), reported use of inhaled corticosteroid, total IgE, serum eosinophil cationic protein and an interaction between sex and smoking habits ($n = 1$ each).

The absolute values of FeNO are presented with great heterogeneity in the included studies. Data are presented using several central tendency and dispersion measures (mean, geometric mean, median, interquartile range, standard deviation and 95% and 90% CIs) and in different subgroups (data not shown).

Discussion

This is the first systematic review concerning reference values for exhaled nitric oxide. Fifteen studies met the selection criteria, and the factors and statistical models used to derive reference equations were retrieved. We have observed great variation in the factors and statistical methods used, which prevents an adequate comparison of reference values from different studies. With current published data, the selection of reference equations for FeNO is a difficult task for laboratories and physicians.

Exhaled NO originates primarily in the airway epithelium, produced by inducible NO synthase (13). Airway NO formation is seen already at birth, and the basal NO formation seen in healthy subjects is highly resistant to corticosteroids (21). Because biological NO formation is a complex and energy-consuming process, this suggests that airway NO formation is important in humans and, consequently, should be under tight biological control under normal circumstances. The origin in the airway epithelium indicates that the total surface area of the airway mucosa will be an important determinant for exhaled NO. 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 children (22). Thus, it is logical that age and height were found to be important factors when evaluating FeNO values, as seen for

Table 1. Description of the included studies

Study	Country	Population	*N*	Age	Atopy	Smoking	Analyser
With reference equations							
Dressel 2008 (20)	Germany	Pre-employment preventive medical check-ups	897	34.5 ± 13.0‡	CH	CH	Sievers NOA 280 (General Electric Company, Fairfield, Connecticut, U.S.)
Habib 2009 (21)	Saudi Arabia	Medical students and hospital personnel	138	31.0 ± 12.2‡	T IgE	Q	NOX EVA 4000 (Seres, Aix-en-Provence, France)
Kovesi 2008 (23)	Canada	Children from schools (Grade 4 to 6)	657	10.8 (9.1–12.9)*	Q	Q	EcoMedics CLD 88sp (EcoMedics, Switzerland)
Kovesi 2008 (24)	Canada	Children from schools (Grade 4 to 6)	1135	10.8 (9.1–12.7)*	Q	Q	EcoMedics CLD 88sp
Levesque 2008 (25)	United States	Local university campus	895	22.5 ± 4.5‡	T IgE	SC	Sievers NOA 280
Malmberg 2006 (28)	Finland	Children from local primary and secondary schools	114	11.6 (6.9–15.7) *	SPT	Q	NIOX (Aerocrine AB, Sweden)
Olin 2006 (30)	Sweden	General population§	2200	50.5§	S IgE	Q	NIOX
Olin 2007 (31)	Sweden	General population§	1131	50.3 ± 13.8‡	S IgE	Q	NIOX
Taylor 2007 (34)	New Zealand	Dunedin Health and Development Study Cohort	895	32¶	SPT T IgE	Q	LR 2000 (Logan Research, UK)
With tables of limit values							
Buchvald 2005 (19)	The Netherlands, Italy, United States	Children from kindergartens and schools	405	4–17†	Q	Q	NIOX
Kim 2010 (22)	South Korea	Hospital employees and medical students	166	M: 33.2 ± 12.1‡ F: 32.2 ± 7.9‡	SPT	Q	Sievers NOA 280i
Liu 2009 (26)	Taiwan	Volunteers from a general health check-up	249	45.3 ± 12.2‡	CH	Q	Sievers NOA 280i
Maestrelli 2007 (27)	Italy	Occupational health clinic	122	40 ± 1.0 ‡	NR	NR	NIOX
Matsunaga 2010 (29)	Japan	General population	240	39.4 ± 13.6‡	CH	CH	NIOX Mino (Aerocrine AB, Sweden)
Travers 2007 (35)	New Zealand	General population	524	56.2 ± 12.9‡	SPT	CH	NIOX

*Median (interquartile range).
†Range.
‡Mean ± standard deviation.
§Weighted mean.
¶All individuals have 32 years (cohort study).
NR, not reported; Q, questionnaire; S IgE, specific immunoglobulin E; T IgE, total immunoglobulin E; SPT, skin prick tests; CH, clinical history; SC, serum cotinine.

The Clinical Respiratory Journal (2013) • ISSN 1752-6981

Table 2. Description of statistical models of the included studies

Study	Model	Dependent variable	Factors	R^2
With reference equations				
Dressel 2008 (20)	MLR (GLM)	Log(FeNO)	Height, smoking, asthma, respiratory tract infection	NR
Habib 2009 (21)	SLR and MLR	FeNO	Bodyweight, BMI	NR
Kovesi 2008 (23)	NR	FeNO	Age, Race, FVC	
Kovesi 2008 (24)	Univariate and MLR	FeNO	Age, height, atopy, asthma, healthy	NR
Levesque 2008 (25)	Univariate and MLR	Log(FeNO)	T IgE, Sex, current URI symptoms, ECP	T IgE: 15% Sex: 21% Current URI symptoms: 25% ECP: 26%
Malmberg 2006 (28)	Analysis of covariance (ANCOVA)	Log10(FeNO)	Age, height, weight, BSA	Height: 17% Age: 15% Weight: 16% BSA: 16%
Olin 2006 (32)	MLR (GLM)	Log(FeNO)	Age, height, atopy, weight, smoking, asthma symptoms in the last month, medical diagnosis of asthma, reported use of ICS	NR
Olin 2007 (33)	MLR (GLM)	FeNO	Age, height, atopy	12%
Taylor 2007 (34)	SLR and MLR	Log(FeNO)	Atopy, smoking, sex, sex*smoker (smoked day of test), sex*smoker (not smoked day of test)	33%
With tables of limit values				
Buchvald 2005 (19)	MLR (backward stepwise)	Ln(FeNO)	Age	NR
Kim 2010 (22)	MLR	Log(FeNO)	Male gender, atopy	NR
Liu 2009 (26)	MLR	Ln(FeNO)	Age, FVC	NR
Maestrelli 2007 (27)	Stepwise regression	Ln(FeNO)	Weight	NR
Matsunaga 2010 (29)	Regression tree	Log(FeNO)	No significant factors	NR
Travers 2007 (35)	MLR	Log(FeNO)	Height, atopy, smoking, sex, asthma	NR

*Multiplication symbol.

Ln, natural logarithm; Log10, decadic logarithm; Log, unknown logarithmic base; FeNO, fraction of exhaled nitric oxide; MLR, multiple linear regression; GLM, general linear model; SLR, simple linear regression; NR, not reported; T IgE, total immunoglobulin E;.BMI, body mass index; FVC, forced vital capacity; URI, Upper Respiratory Infection; ECP, eosinophil cationic protein; BSA, Body Surface Area; ICS, inhaled corticosteroid.

other lung function parameters, and it is especially important to take these factors into account when evaluating FeNO of growing children. Furthermore, Gelb *et al*. (23) have recently shown that FeNO and alveolar NO, but not bronchial NO flux, increase with age in adults, especially above 60 years. This is suggested to be because of the reduced lung diffusing capacity for NO (and carbon monoxide) seen in elderly, leading to less uptake of bronchial NO in the alveolar tract. Because the effect of age is stronger below 18 years and above 60 years and the effect of height is stronger in children, the regression needs to be nonlinear if the population under study includes all ages. Some studies have reported conflicting results on the influence of sex on FeNO values (24, 25). In the 15 included studies, only two had sex as a significant factor (25, 26). However, FeNO would be expected to be higher in men than women, regardless of e.g. height, because men have a larger anatomic dead space volume in relation to bodyweight [see Pedroletti *et al*.(22)].

Few studies included non-Caucasian subjects (19, 27, 28). As the effect of race on FeNO values is far from established (29), current data are vastly inadequate to allow conclusions concerning people of other genetic

background. There is evidence that race and ethnicity may play an important role in lung function prediction (32).

Most of the studies included did not assess atopy and smoking habits with objective methods. For the definition of reference values for FeNO, these are essential variables and their assessment using questionnaire data is insufficient.

In addition to individual-specific factors, several behavioural and environmental factors have been pointed out as influencing FeNO, such as rhinovirus infections (33), allergen exposure (34, 35), physical exercise (36, 37), ozone exposure (38, 39) and air pollution (40). In fact, the low percentage of variance explained by the reported models possibly reveals the difficulty of determining the effect of different exogenous factors and their combination on FeNO. For example, the effect of atopy cannot easily be captured in a single factor, because atopy may result in an increase in FeNO of anywhere between zero and several hundred parts per billion (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 gender on expected normal FeNO values.

A source of variation of the reference equations published may be the use of different FeNO analysers or calibration procedures (41), even though all 15 studies reported that they were following the ATS/ERS FeNO measurement guidelines (18).

FeNO reference values should be further refined in the future, perhaps in ways similar to those recently reviewed by Stanojevic *et al*. (14) for lung function measurements. 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 FeNO reference equation studies. A large US dataset meeting many of the earlier criteria was recently published as part of the National Health and Nutrition Examination Survey 2007–2008 and 2009–2010 (42).

The definition and future use of specific guidelines on how to report studies on reference values may contribute to the standardization of reports. Published FeNO recommendations (18, 43) are helpful in the standardization of the measurement, but not in the standardization of how the methods are described.

The interpretation of FeNO 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. The per cent predicted of the reference value is now a standard transformation in most lung function laboratories, and z-scores are increasingly suggested (14). We suggest the use of a similar approach when interpreting FeNO values, either by using the per cent predicted of the reference value or z-scores. Further research is needed to clarify this proposal. Nevertheless, the individual factors taken into consideration will be an important step to improve the interpretation of FeNO values. Such factors are easily accessible at the clinic and incorporating them will require very little extra effort. Most importantly, if reference equations are used, clinical cut-offs can be generalized across age groups and genetic backgrounds.

In conclusion, several reference equations are already available, albeit mainly for Caucasians. These can be used in clinical practice, provided that the study characteristics (population, sampling, objective measures) are taken into consideration when such an equation is used for the interpretation of a FeNO value. The published equations differ considerably in terms of individual-specific factors that have an effect on FeNO values, and there is little standardization of the method description in the studies, both on the statistical and technical side. We suggest that the methodology and reporting on normal FeNO values and the corresponding reference equations should be standardized and that 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.

References

1. Taylor DR, Pijnenburg MW, Smith AD, De Jongste JC. Exhaled nitric oxide measurements: clinical application and interpretation. Thorax. 2006;61(9): 817–27.
2. Berkman N, Avital A, Breuer R, Bardach E, Springer C, Godfrey S. Exhaled nitric oxide in the diagnosis of asthma: comparison with bronchial provocation tests. Thorax. 2005;60(5): 383–8.
3. Cardinale F, de Benedictis FM, Muggeo V, Giordano P, Loffredo MS, Iacoviello G, Armenio L. Exhaled nitric oxide, total serum IgE and allergic sensitization in childhood asthma and allergic rhinitis. Pediatr Allergy Immunol. 2005;16(3): 236–42.
4. Janson C, Kalm-Stephens P, Foucard T, Norbäck D, Alving K, Nordvall SL. Exhaled nitric oxide levels in school children in relation to IgE sensitisation and window pane condensation. Respir Med. 2005;99(8): 1015–21.

5. Syk J, Undén AL, Alving K. Relationship between exhaled nitric oxide and IgE sensitisation in patients with asthma: influence of steroid treatment. Clin Respir J. 2009;3(3): 143–51.
6. Mattes J, Storm Van's Gravesande K, Reining U, Alving K, Ihorst G, Henschen M, Kuehr J. NO in exhaled air is correlated with markers of eosinophilic airway inflammation in corticosteroid-dependent childhood asthma. Eur Respir J. 1999;13(6): 1391–5.
7. Choi J, Hoffman LA, Rodway GW, Sethi JM. Markers of lung disease in exhaled breath: nitric oxide. Biol Res Nurs. 2006;7(4): 241–55.
8. Van Den Toorn L, Overbeek S, De Jongste J, Leman K, Hoogsteden H, Prins J. Airway inflammation is present during clinical remission of atopic asthma. Am J Respir Crit Care Med. 2001;164(11): 2107–13.
9. Kharitonov SA, Donnelly LE, Montuschi P, Corradi M, Collins JV, Barnes PJ. Dose-dependent onset and cessation of action of inhaled budesonide on exhaled nitric oxide and symptoms in mild asthma. Thorax. 2002;57(10): 889–96.
10. Silkoff PE, McClean P, Spino M, Erlich L, Slutsky AS, Zamel N. Dose-response relationship and reproducibility of the fall in exhaled nitric oxide after inhaled beclomethasone dipropionate therapy in asthma patients. Chest. 2001;119(5): 1322–8.
11. Silkoff PE, McClean PA, Slutsky AS, Caramori M, Chapman KR, Gutierrez C, Zamel N. Exhaled nitric oxide and bronchial reactivity during and after inhaled beclomethasone in mild asthma. J Asthma. 1998;35(6): 473–9.
12. Lim KG, Mottram C. The use of fraction of exhaled nitric oxide in pulmonary practice. Chest. 2008;133(5): 1232–42.
13. Alving K, Malinovschi A. Basic aspects of exhaled nitric oxide, in European Respiratory Monograph 2010.
14. Stanojevic S, Wade A, Stocks J. Reference values for lung function: past, present and future. Eur Respir J. 2010;36(1): 12–9.
15. Daly L, Bourke GJ. *Interpretation and Uses of Medical Statistics*. Oxford, Wiley-Blackwell, 2000.
16. Sackett DL, Haynes RB. The architecture of diagnostic research. BMJ. 2002;324(7336): 539–41.
17. Smith AD, Cowan JO, Taylor DR. Exhaled nitric oxide levels in asthma: personal best versus reference values. J Allergy Clin Immunol. 2009;124(4): 714–718.e4.
18. ATS/ERS. 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(8): 912–30.
19. Levesque MC, Hauswirth DW, Mervin-Blake S, Fernandez CA, Patch KB, Alexander KM, Allgood S, McNair PD, Allen AS, Sundy JS. Determinants of exhaled nitric oxide levels in healthy, nonsmoking African American adults. J Allergy Clin Immunol. 2008;121(2): 396–402 e3.
20. Maestrelli P, Ferrazzoni S, Visentin A, Marian E, Dal Borgo D, Accordino R, Fabbri LM. Measurement of exhaled nitric oxide in healthy adults. Sarcoidosis Vasc Diffuse Lung Dis. 2007;24(1): 65–9.
21. Lundberg J, Weitzberg E, Lundberg J, Alving K. Nitric oxide in exhaled air. Eur Respir J. 1996;9(12): 2671.
22. Pedroletti C, Högman M, MeriLäinen P, Nordvall LS. Nitric oxide airway diffusing capacity and mucosal concentration in asthmatic schoolchildren. Pediatr Res. 2003;54(4): 496–501.
23. Gelb AF, George SC, Camacho F, Fraser C, Flynn Taylor C, Shakkottai S. (2011). Increased Nitric Oxide Concentrations in the Small Airway of Older Normal Subjects. CHEST Journal, 139(2), 368-375. doi: 10.1378/chest.10-1157
24. Olin A-C, Bake B, Torén K. Fraction of exhaled nitric oxide at 50 mL/s: reference values for adult lifelong never-smokers. Chest. 2007;131(6): 1852–6.
25. Taylor DR, Mandhane P, Greene JM, Hancox RJ, Filsell S, McLachlan CR, Williamson AJ, Cowan JO, Smith AD, Sears, MR. Factors affecting exhaled nitric oxide measurements: the effect of sex. Respir Res. 2007;8: 82.
26. Travers J, Marsh S, Aldington S, Williams M, Shirtcliffe P, Pritchard A, Weatherall M, Beasley, R. Reference ranges for exhaled nitric oxide derived from a random community survey of adults. Am J Respir Crit Care Med. 2007;176(3): 238–42.
27. Kovesi T, Dales R. Exhaled nitric oxide and respiratory symptoms in a community sample of school aged children. Pediatr Pulmonol. 2008;43(12): 1198–205.
28. Kovesi T, Kulka R, Dales R. Exhaled nitric oxide concentration is affected by age, height, and race in healthy 9- to 12-year-old children. Chest. 2008;133(1): 169–75.
29. Sandrini A, Taylor DR, Thomas PS, Yates DH. (2010). Fractional exhaled nitric oxide in asthma: an update. Respirology. 15(1): 57–70.
30. Olin A-C, Rosengren A, Thelle DS, Lissner L, Bake B, Torén K. Height, age, and atopy are associated with fraction of exhaled nitric oxide in a large adult general population sample. Chest. 2006;130(5): 1319–25.
31. Olin A-C, Bake B, Torén K. Fraction of exhaled nitric oxide at 50 mL/s: reference values for adult lifelong never-smokers. Chest. 2007;131(6): 1852–6.
32. Kumar R, *et al*. Genetic ancestry in lung-function predictions. N Engl J Med. 2010;363(4): 321–30.
33. de Gouw H, Grunberg K, Schot R, Kroes A, Dick E, Sterk P. Relationship between exhaled nitric oxide and airway hyperresponsiveness following experimental rhinovirus infection in asthmatic subjects. Eur Respir J. 1998;11(1): 126–32.
34. Baraldi E, Carra S, Dario C, Azzolin N, Ongaro R, Marcer G, Zacchello F. Effect of natural grass pollen exposure on exhaled nitric oxide in asthmatic children. Am J Respir Crit Care Med. 1999;159(1): 262–6.
35. Bodini A, Peroni D, Loiacono A, Costella S, Pigozzi R, Baraldi E, Boner AL, Piacentini GL. Exhaled nitric oxide daily evaluation is effective in monitoring exposure to

relevant allergens in asthmatic children. Chest. 2007;132(5): 1520–5.
36. Gabriele C, Pijnenburg MW, Monti F, Hop W, Bakker ME, de Jongste JC. The effect of spirometry and exercise on exhaled nitric oxide in asthmatic children. Pediatr Allergy Immunol. 2005;16(3): 243–7.
37. Verges S, Tonini J, Flore P, Favre-Juvin A, Levy P, Wuyam B. Exhaled nitric oxide in single and repetitive prolonged exercise. J Sports Sci. 2006;24(11): 1157–63.
38. Nickmilder M, de Burbure C, Carbonnelle S, Dumont X, Bernard A, Derouane A. Increase of exhaled nitric oxide in children exposed to low levels of ambient ozone. J Toxicol Environ Health A. 2007;70(3–4): 270–4.
39. Olin AC, Stenfors N, Toren K, Blomberg A, Helleday R, Ledin MC, Ljungkvist G, Ekman A, Sandstrom T. Nitric oxide (NO) in exhaled air after experimental ozone exposure in humans. Respir Med. 2001;95(6): 491–5.
40. Delfino RJ, Staimer N, Gillen D, Tjoa T, Sioutas C, Fung K, George SC, Kleinman MT. Personal and ambient air pollution is associated with increased exhaled nitric oxide in children with asthma. Environ Health Perspect. 2006;114(11): 1736–43.
41. Alving K, Janson C, Nordvall L. Performance of a new hand-held device for exhaled nitric oxide measurement in adults and children. Respir Res. 2006;7: 67.
42. (NCHS)., C.f.D.C.a.P.C.N.C.f.H.S. *National Health and Nutrition Examination Survey Data*. 2011. Available at: http://www.cdc.gov/nchs/nhanes.htm (accessed 21 Nov 2011).
43. ATS. Recommendations for standardized procedures for the on-line and off-line measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide in adults and children-1999. Am J Respir Crit Care Med. 1999;160(6): 2104–17.