Reference range values of fractional exhaled nitric oxide in healthy Arab adult males

Syed S. Habib, MBBS, FCPS, ***Abdullah A. Abba,*** FRCP (Glas), FRCPI, ***Mohammed A. Al-Zoghaibi,*** BSc, PhD, ***Mirza M. Subhan,*** BSc, PhD.

ABSTRACT

الأهداف: تحديد مرجع للقيم الطبيعية لأكسيد النيتريك المزفور FENO في الأشخاص الذكور الأصحاء والغير مدخنين ومعرفة علاقتها بالعمر، والطول، والوزن، وكتلة الجسم (BMI).

الطريقة: تم إجراء هذه الدراسة الميدانية في قسمي علم وظائف الأعضاء والباطنية– كلية الطب ومستشفى الملك خالد– جامعة الملك سعود – الرياض خلال الفترة من سبتمبر 2007 إلى أغسطس 2008. كانت العينات من أشخاص سعوديين، ليس لديهم حساسية موروثة وغير مدخنين. تم حساب أكسيد النيتريك المزفور FENO باستخدام آلية النفس الواحد طبقاً لدليل جمعية الصدر الأمريكية (ATS).

النتائج: درسنا 121 شخص، متوسط العمر 31.00±12.24 عام متوسط كتلة الجسم BMI 27.23±6.64 و FEV1/FVC 85% مابين 81%–92%، كان مدى أكسيد النيتريك المزفور FENO بين 7.66 جزء لكل بليون (ppb) و 46.6ppb (متوسط 22.79±8.13)، أكثر من 84% من الأشخاص سجلوا مستوى أقل من 30ppb> وأكثر من 95% حصلوا على أقل من 40ppb>. تلازم أكسيد النيتريك المزفور FENO سلبياً مع وزن الجسم (p=0.001)، r=0.3888 وكتلة الجسم p=0.009، BMI r=0.238. لم يتم ملاحظة أي تلازم بين أكسيد النيتريك المزفور FENO، ونسبة FEV1/FVC، والعمر، والطول.

خاتمة: إن القيم المرجعية لأكسيد النيتريك المزفور FENO للأشخاص السعوديين البالغين والغير مدخنين وليس لديهم حساسية وراثية هي بين 7.66 و 46.6ppb متوسط(22.79±8.13) مشابه للسكان الآخرين. يتلازم أكسيد النيتريك المزفور FENO سلبياً مع كتلة الجسمBMI ووزنه.

Objectives: To determine the reference values of the fraction of exhaled nitric oxide (FENO) among healthy, non-smoking male adults and its correlation with age, height, weight, and body mass index (BMI).

Methods: This cross-sectional study was conducted at the Departments of Physiology and Medicine, College of Medicine and King Khalid University Hospital, King Saud University, Riyadh, from September 2007 to August 2008 on healthy non-atopic, non-smoking male Saudi subjects. The FENO was measured online using the single-breath technique according to recent guidelines of the American Thoracic Society (ATS).

Results: We studied 121 subjects with a mean age of 31.00±12.24 years, BMI of 27.23±6.64, and FEV1/FVC 85% (81-92%). The FENO ranged between 7.66 parts per billion (ppb) and 46.6 ppb (mean 22.79±8.13), with >84% of subjects recording levels <30 ppb and >95% with levels <40 ppb. The FENO negatively correlated with body weight (r=0.3888, p=0.001) and BMI (r=0.238, p=0.009). No correlation was observed between FENO, FEV1/FVC ratio, age, and height.

Conclusion: The reference values of FENO for non-smoking, non-atopic male Saudi adults fall between 7.66 and 46.6 ppb (mean 22.79±8.13), similar to other populations. The FENO negatively correlates with body weight and BMI.

Saudi Med J 2009; Vol. 30 (11): 1395-1400

From the Departments of Physiology (Habib, Al-Zoghaibi) and Medicine (Abba), College of Medicine and King Khalid University Hospital, King Saud University, Riyadh, Kingdom of Saudi Arabia and the Department of Physiology (Subhan), College of Medicine & Medical Sciences, Arabian Gulf University, Manama, Kingdom of Bahrain.

Received 7th July 2009. Accepted 13th October 2009.

Address correspondence and reprint request to: Dr. Syed S. Habib, Associate Professor, Department of Physiology (29), College of Medicine, PO Box 2925, King Saud University, Riyadh 11461, Kingdom of Saudi Arabia. Tel. +966 (1) 4671604. Fax. +966 (1) 4672567. E-mail: shahidhabib44@hotmail.com

Nitric oxide (NO) is a molecule involved in the regulation of bronchial and vascular tone, inflammation, and neurotransmission.[1] Since the demonstration of the presence of NO in exhaled air by Gustafsson et al[2] in 1991, there have been numerous publications in the world literature showing that the fraction of exhaled nitric oxide (FENO) is high in many respiratory diseases,[2-4] for example, in bronchial asthma, the level has been shown to be higher than in the general population, and it increases with exacerbations and decreases with anti-inflammatory treatment.[5] We have also shown recently that FENO is high in stable chronic obstructive pulmonary disease.[6] Cheaper and more portable NO analyzers are now readily available and are increasingly being used for not only the diagnosis but also the assessment of asthma control. Furthermore, the American Thoracic Society (ATS) and European Respiratory Society have jointly published recommendations for the procedure of measurement of both exhaled and nasal NO to allow for the comparison of results from various centers.[7] A number of factors including age, atopy, anthropometric differences, and probably gender and race have been established as affecting the values of exhaled NO. Given the importance of FENO in the assessment of airway inflammation and its potential use in clinical practice, there is a need to establish a reference for different populations to aid in the interpretation of measured values. There are only a few reports from other countries regarding reference values of FENO among targeted adult populations.[8-12] There are no reports on the values of FENO among healthy adults in this region. The aim of the present study was to establish reference values of FENO among the healthy male Saudi adult population using the recent international guidelines of the ATS and to assess the association of age, height, weight, and body mass index.

Methods. This cross-sectional study was conducted at the Departments of Physiology and Medicine of the College of Medicine and King Khalid University Hospital, King Saud University, Riyadh, Saudi Arabia from September 2007 to August 2008. Written informed consent was obtained from all subjects and the project was approved by the College of Medicine Ethics Review board. All subjects completed a brief questionnaire. They were included in the study if they were non-smokers, had no recent or current upper respiratory tract infection, were not on any medications, and had neither atopy nor clinical manifestations of allergic diseases. In addition, all subjects included in the study had normal spirometry and serum IgE levels. Patients were excluded from the study if they had physician diagnosis of respiratory disease, symptoms of respiratory disease in the last one year, or were on inhaled medications. One hundred and thirty-eight subjects were initially recruited consisting of medical students and hospital personnel. Seventeen subjects were excluded due to either inability to give definitive answers to the questionnaire, or they could not perform the test properly according to ATS guidelines.

Exhaled nitric oxide measurements. The FENO measurements were performed according to the present recommendations of the ATS using a NOX EVA 4000 chemiluminescence analyzer (Seres, France) with a sensitivity of one part per billion (ppb). All subjects were asked to refrain from eating, drinking, and strenuous exercise for 2 hours before FENO measurement. The history of recent meals was also recorded to avoid any alteration in results by nitrate containing foods. As an additional precaution, all tests were performed at the same time of the day between 0900 hours and 1100 hours to minimize possible circadian effects. Using online visual monitoring, the subjects were asked to inhale from residual volume to total lung capacity (TLC) and then performed a slow expiratory vital capacity maneuver with a constant standardized expiratory flow rate of 0.05 L/sec (± 10%) resulting in an expiration time of approximately 20 seconds, into a Teflon cylinder connected to 3-mm Teflon tubing, without clipping the nose. To exclude nasal NO contamination an expiratory resistance of 10-20 cm H_2O was applied. This expiratory resistance was measured by a special pressure sensor (SAMBA 3200 pressure measurement system) connected to restricted breathing configuration set up (Samba Sensors, Vastra Frolunda, Sweden). The subjects inspired from NO free air and expired in restricted-breath configuration set up.

The expiratory flow rate was measured by a data acquisition system BIOPAC MP-100 (Biopac Systems Inc, USA). Plateau levels of FENO against time were determined and expressed as parts per billion (ppb). Mean exhaled NO concentrations were determined between 5 and 15 seconds after start of the expiration. Three successive recordings were made at one-minute intervals, and the mean was used in analysis. To ensure standardization and reproducibility, the acceptable variation between the tests was kept to less than 10%. The NO measurement set up was calibrated before each test using a standard NO calibration gas.

Disclosure. This study was supported by a grant from King Abdulaziz City for Science and Technology, Riyadh, Kingdom of Saudi Arabia (Grant no. ARP-24-64).

Ventilatory function parameters. Spirometric procedures were performed after FENO measurements. Forced expiratory volume in one second (FEV1), forced vital capacity (FVC), FEV1/FVC (%ratio), peak expiratory flow (PEF), FEF25, FEF50, and FEF75 were measured using an electronic spirometer (Vitalograph, Ennis Co. Clare, Ireland) and which was calibrated daily. All recordings were made in a sitting position. At least 3 readings were obtained and the best of 3 was taken as the final result.

Statistical analysis. The data were analyzed by the computer software program the Statistical Package for Social Sciences (SPSS Version 11). Data were expressed as mean ± SD for continuous variables and as percentages for categorical variables. Frequency distribution and cumulative percent were determined for FENO. We calculated Pearson's correlation coefficient to see the relation of FENO with other parameters. Multiple linear regression was carried out to find the predictor variables for FENO. A p-value of ≤0.05 was taken as statistically significant and all tests were 2-tailed.

Table 1 - Clinical and demographic characteristics of subjects.

Clinical and demographic characteristics	Mean ± SD	Median	Range
Age (years)	31.0 ± 12.2	26.00	19.0 - 64.0
Height (cm)	171.8 ± 8.3	172.00	149.0 - 188.0
Weight(kg)	80.2 ± 18.4	76.50	53.2 - 148.9
BMI	27.2 ± 6.5	25.81	18.2 - 45.9
FEV1/FVC (%)	85.0	85.00	81.0 - 92.0
FENO (ppb)	22.8 ± 8.1	21.30	7.7 - 46.6

BMI - body mass index, FEV1 - forced expiratory volume in the first second, FVC - forced vital capacity, FENO - fraction of exhaled nitric oxide, ppb - parts per billion

Table 2 - Cumulative percentage distribution according to different ranges of FENO.

FENO ranges	Cumulative Percent
<10	5.0
10.01 - 15	13.2
15.01 - 20	38.8
20.01 - 25	65.3
25.01 - 30	84.3
30.01 - 35	90.1
35.01 - 40	95.9
40.01 - 45	99.2
45.01 - 50	100.0

FENO = fraction of exhaled nitric oxide

Results. All study subjects were males and non-smokers at the time of recruitment. One hundred and thirty-eight subjects were initially recruited consisting of medical students and hospital personnel out of whom 121 subjects were finally selected. The clinical and demographic characteristics of the subjects are shown in **Table 1**. Measured FENO ranged between 7.66 ppb to 46.60 ppb with a mean of 22.79 ppb ± 8.13. The cumulative percentage distribution according to different ranges of FENO is shown in **Table 2** and the distribution of FENO according to different ranges of FENO in shown in **Figure 1**. Significant negative correlation was observed between the measured FENO and weight and body mass index, as shown in **Figures 2 & 3**. There was no correlation noted between age and height and measured FENO (**Table 3**). The prediction equation for weight and BMI was (FENO = 47.096 - 0.119 x weight) and (FENO = 31.541 - 0.289 x BMI).

Discussion. The primary objective of this study was to define FENO levels in healthy, male Saudi subjects measured in accordance with the current recommended standards. Until now, only a few studies examined the values of FENO in more than 50 adults using current standard methods.[8-11] Of these studies, the one by Olivieri et al[10] was designed, like our study, to determine reference values in non-atopic, non-smoking subjects. Their figures were much lower than ours; the lower and upper limits being 3.8 and 19.7 ppb. The values we obtained are in consonance with those reported by Travers et al[9] in a study of a large number of white subjects in New Zealand. The authors reported a geometric mean of 17.9 ppb with a confidence interval for an individual prediction for normal subjects of 7.8 to 41.1 ppb. Reference values for exhaled nitric

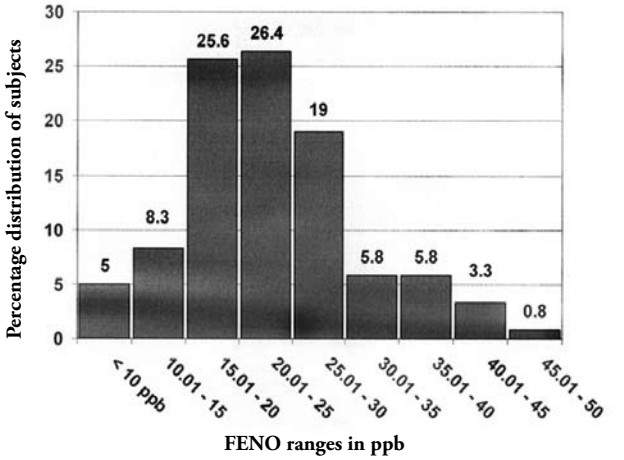

Figure 1 - Data distribution histogram of fraction of exhaled nitric oxide (FENO) according to different ranges of FENO.

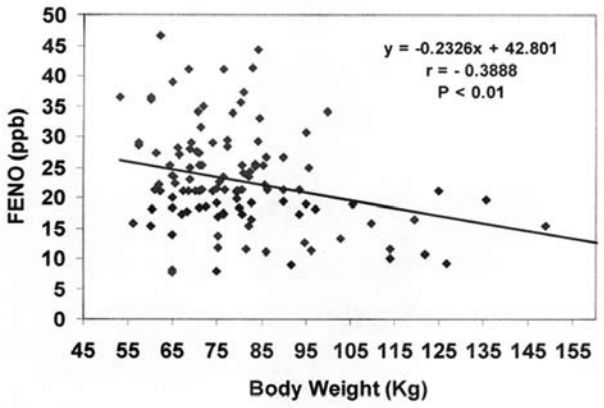

Figure 2 - Relationship between fraction of exhaled nitric oxide (FENO) and body weight.

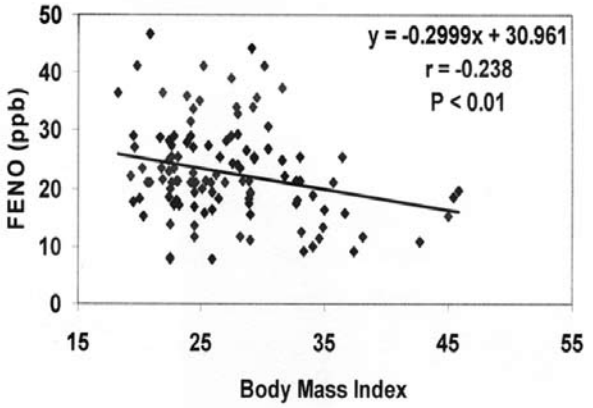

Figure 3 - Relationship between fraction of exhaled nitric oxide (FENO) and body mass index.

Table 3 - Pearson's correlation between FENO, age, height, weight, BMI and FEV1/FVC.

Parameters	Age	Height	Weight	BMI	FENO	FEV1/FVC
Age	1.000	-0.379*	0.036	0.209‡	-0.091	0.008
Height	-.379*	1.000	0.222†	-0.234†	-0.092	-0.272
Weight	0.036	0.222†	1.000	0.882*	-0.290*	0.052
BMI	0.209‡	-0.234†	0.882*	1.000	-0.238†	0.140
FENO	-0.091	-0.092	-0.290*	-.238	1.000	-0.064
FEV1/FVC	0.008	-0.272	0.052	0.140	-0.064	1.000

*Correlation is significant at the 0.001 level (2-tailed). †Correlation is significant at the 0.01 level (2-tailed).
‡Correlation is significant at the 0.05 level (2-tailed). BMI - body mass index; FEV1 - forced expiratory volume in the first second; FVC - forced vital capacity, FENO - fraction of exhaled nitric oxide, ppb - parts per billion

oxide (REVENO) study[10] showed lower mean values than ours (10.8 ppb) with males having higher values than females (11.7 versus 9.9 ppb).[10] However, Olin et al[8] reported higher range values than ours (24.0 to 54.0 ppb) with no gender difference. The differences observed underline the importance of confounding factors in the measurement of FENO. While smoking habits, gender, anthropometric differences, presence of asthma and/or atopy may be taken into consideration, other factors might be difficult to exclude. Recently, a positive correlation between FENO and dietary consumption of fats in children was reported.[13] Taking dietary differences into consideration might have been difficult in our study. However, we advised our subjects to refrain from eating, drinking, and strenuous exercise for at least 2 hours before FENO measurement.

Although measurement of FENO has been shown to be free from diurnal and day-day variation,[14] we took the additional precaution of making all measurements between 0900 hours and 1100 hours. Another factor contributing to the discrepancies in the figures may be the differences in the NO analyzers used. Significant differences sufficient to explain the discrepancies have been reported between analyzers of different manufacturers.[15] Although carried out in children, a study by Kovesi et al[16] demonstrated significantly higher levels of FENO among Asians compared to whites. In their study, black children had higher figures than whites but lower than Asians. Similarly, Buchvald et al[17] observed that nonwhite subjects tended to have a higher FENO than whites, and Wong et al[18] also noted that Chinese subjects had higher figures than whites. Our findings of higher FENO levels in Saudi subjects may support the view that Asian subjects have higher FENO levels than Caucasians. No genetic explanation could be found for these differences by Leung et al.[19]

Our study found no correlation between age, and height, and FENO levels. This is in contradiction with the study by Travers et al[9] of an adult population in which they found a weak correlation between height and FENO with a correlation coefficient of 0.16 (p=0.024). However, there was no significant correlation between age and FENO. Taylor et al[20] in a study of 895 adults aged 32 years, revealed significant differences in FENO in relation to height in unadjusted analysis. Adjusting for gender, however, made height insignificant. Another study from Italy by Olivieri et al[10] involving 204 adults also did not find the FENO

levels to be correlated with age (r = 0.1, p=0.21), lung function or anthropometric values in either males or females. In children, where there is a relationship between age and body size, a clear relationship has been observed between age and FENO.[18,21] The study by Olin et al[22] is the first to observe an independent and positive association between age and FENO in adults. The oldest group (>64 years) had a 40% higher FENO value as compared to the youngest group (35-44 years). The present study identified a negative correlation between both weight and BMI to FENO. Markers of systemic inflammation are known to be increased in obese patients, but no clear relationship has been established between obesity and airway inflammation.[23] Among other things, obesity induces systemic oxidative stress in part through increased production of reactive oxygen species in adipose tissue. It is hypothesized that the lung serves as a target organ for this oxidative stress. This is manifested as increased oxidation of airway NO into nitrate and reactive nitrogen species and hence reduction of NO bioavailability and exhaled NO levels. It has been shown in asthma patients that BMI and plasma ratio of leptin/adiponectin is associated with reduced exhaled NO and that BMI is associated with increased exhaled 8-isoprostanes. No relationship was found in this study between BMI and FENO in control subjects.[24] Maestrelli et al,[25] on the other hand, found that FENO increased significantly with body size and spirometric indices. In a stepwise regression analysis, body weight was the only variable included in the model (r=0.36, p<0.0001) and explained gender differences in FENO. Similarly, Tsang et al[26] found significant correlation between FENO and weight (r=0.34, p<0.001).[26] Among Chinese children, there was no significant correlation between weight, BMI, body surface area, and height.[18] Our study is therefore, the first to show a negative correlation. This report is supported by the findings of Maniscalco et al[27] who observed that FENO is consistently reduced in severe obesity, and it is restored after weight reduction.[27] Our study is limited by relatively small sample size and inclusion of only male subjects. Further studies with a larger number of subjects and both genders are needed to be able to define exactly the relationship between these parameters in normal adults especially the relationship between obesity, asthma, and FENO.

In conclusion, the reference values of FENO for non-smoking, non-atopic male Saudi patients fall between 7.66 ppb and 46.6 ppb (mean 22.79 ± 8.13), a figure slightly higher than other populations. A negative correlation was observed between FENO and BMI and body weight in this normal population. Further studies are needed to define the figures for females in this country and to elucidate the relationship between BMI and FENO.

Acknowledgment. *The sponsors have no interest and were not involved in the development, conduct, and writing of the manuscript. We are thankful to Dr. Shahab Shaikh and Syed Azeem Shah for assistance in FENO measurements.*

References

1. Ricciardolo FL, Sterk PJ, Gaston B, Folkerts G. Nitric oxide in health and disease of the respiratory system. ***Physiol Rev*** 2004; 84: 731-765. Review.
2. Gustafsson LE, Leone AM, Persson MG, Wiklund NP, Moncada S. Endogenous nitric oxide is present in the exhaled air of rabbits, guinea pigs and humans. ***Biochem Biophys Res Commun*** 1991; 181: 852-857.
3. Taylor DR, Pijnenburg MW, Smith AD, De Jongste JC. Exhaled nitric oxide measurements: clinical application and interpretation. ***Thorax*** 2006; 61: 817-827.
4. Barroso NC, Perez-Yarza EG, Prado OS, Bover CR, Gartner S, Murua JK. Exhaled nitric oxide in children: a non-invasive marker of airway inflammation. ***Archivos de Broncopeumologia*** 2008; 44: 41-51.
5. Kharitonov SA, Barnes PJ. Biomarkers of some pulmonary diseases in exhaled breath. ***Biomarker***s 2002, 7: 1-32.
6. Beg MF, Alzoghaibi MA, Abba AA, Habib SS. Exhaled nitric oxide in stable chronic obstructive pulmonary disease. ***Ann Thorac Med*** 2009; 4: 65-70.
7. American Thoracic Society; European Respiratory Society. 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.
8. Olin AC, Bake B, Torén K. Fraction of exhaled nitric oxide at 50 mL/s: reference values for adult lifelong never-smokers. ***Chest*** 2007; 131: 1852-1856.
9. Travers J, Marsh S, Aldington S, Williams M, Shirtcliffe P, Pritchard A, 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.
10. Olivieri M, Talamani G, Corradi M, Perbellini L, Mutti A, Tantucci C, Malerba M. Reference values for exhaled nitric oxide (REVENO) study. ***Respir Res*** 2006; 7: 94.
11. Olin AC, Andersson E, Andersson M, Granung G, Hagberg S, Torén K. Prevalence of asthma and exhaled nitric oxide are increased in bleachery workers exposed to ozone. ***Eur Respir J*** 2004; 23: 87-92.
12. Grasemann H, Storm van's Gravesande K, Buscher R, Drazen JM, Ratjen F. Effects of sex and of gene variants in constitutive nitric oxide synthases on exhaled nitric oxide. ***Am J Resp Crit Care Med*** 2003; 167: 1113-1116.
13. Cardinale F, Tesse R, Fucilli C, Loffredo MS, Iacoviello G, Chinellato I, et al. Correlation between exhaled nitric oxide and dietary consumption of fats and antioxidants in children with asthma. ***J Allergy Clin Immunol*** 2007; 119: 1268-1270.
14. Kharitonov SA, Gonio F, Kelly C, Meah S, Barnes PJ. Reproducibility of exhaled nitric oxide measurements in healthy and asthmatic adults and children. ***Eur Respir J*** 2003; 21: 433-438.
15. Borrill Z, Clough D, Truman N, Morris J, Langley S, Singh D. A comparison of exhaled nitric oxide measurements performed using three different analysers. ***Respir Med*** 2006; 100: 1392-1396.

16. 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: 169-175.
17. Buchvald F, Baraldi E, Carraro S, Gaston B, De Jongste J, Pijnenburg MW, et al. Measurements of exhaled nitric oxide in healthy subjects aged 4 to 17 years. ***J Allergy Clin Immunol*** 2005; 115: 1130-1136.
18. Wong GW, Liu EK, Leung TF, Yung E, Ko FW, Hui DS, et al. High levels and gender difference of exhaled nitric oxide in Chinese schoolchildren. ***Clin Exp Allergy*** 2005; 35: 889-893.
19. Leung TF, Liu EK, Tang NL, Ko FW, Li CY, Lam CW, et al. Nitric oxide synthase polymorphisms and asthma phenotypes in Chinese children. ***Clin Exp Allergy*** 2005; 35: 1288-1294.
20. Taylor RD, Mandhane P, Greene JM, Hancox RJ, Filsell S, McLachlan CR, et al. Factors affecting exhaled nitric oxide measurements: the effect of sex. ***Respir Res*** 2007; 8: 82.
21. Franklin PJ, Taplin R, Stick SM. A community study of exhaled nitric oxide in healthy children. ***Am J Respir Crit Care Me***d 1999; 159: 311-314.
22. Olin AC, Rosengren A, Thelle DS, Lissner L, Bake B, Torren K. Height, age, and atopy are associated with fraction of exhaled nitric oxide in a large adult general population sample. ***Chest*** 2006; 130: 1319-1325.
23. Sutherland TJ, Cowan JO, Young S, Goulding A, Grant AM, Williamson A, et al. The association between obesity and asthma: interactions between systemic and airway inflammation. ***Am J Respir Crit Care Med*** 2008; 178: 469-475.
24. Komakula S, Khatri S, Mermis J, Savill S, Haque S, Rojas M, et al. Body mass index is associated with reduced exhaled nitric oxide and higher exhaled 8- isoprostanes in asthmatics. ***Respir Res*** 2007; 8: 32.
25. Maestrelli P, Ferrazzoni S, Visentin A, Marian E, Dal Borgo D, Accordino R, et al. Measurement of exhaled nitric oxide in healthy adults. ***Sarcoidosis Vasc Diffuse Lung Dis*** 2007; 24: 65-69.
26. Tsang KW, Ip SK, Leung R, Tipoe GL, Chan SL, Shum IH, et al. Exhaled nitric oxide: the effects of age, gender and body size. ***Lung*** 2001; 179: 83-91.
27. Maniscalco M, de Laurentiis G, Zedda A, Faraone S, Giardiello C, Cristiano S, et al. Exhaled nitric oxide in severe obesity: effect of weight loss. ***Respir Physiol Neurobiol*** 2007; 156: 370-373.

Ethical Consent

All manuscripts reporting the results of experimental investigations involving human subjects should include a statement confirming that informed consent was obtained from each subject or subject's guardian, after receiving approval of the experimental protocol by a local human ethics committee, or institutional review board. When reporting experiments on animals, authors should indicate whether the institutional and national guide for the care and use of laboratory animals was followed.