
Effect of bronchoconstriction on exhaled nitric oxide levels in healthy and asthmatic children

Hiromi Tadaki, MD*†; Hiroyuki Mochizuki, MD, PhD*; Reiko Muramatsu, MD*; Satomi Hagiwara, MD*; Satoru Takami, MD*; Takahisa Mizuno, MD*; and Hirokazu Arakawa, MD, PhD*

Background: Exhaled nitric oxide (eNO) has recently been proposed to be a noninvasive marker of airway inflammation in asthma.

Objective: To evaluate the effect of bronchoconstriction by means of methacholine inhalation challenge on levels of eNO in children.

Methods: Spirometry, impulse oscillometry, and eNO measurements were performed before and after methacholine inhalation challenge (bronchoconstriction phase) and after β_2 -agonist inhalation (bronchodilation phase) in 92 children (62 children with asthma, 13 wheezy children, and 17 healthy children).

Results: A significant decrease occurred in the eNO level after methacholine inhalation challenge ($P < .01$). This decrease did not correlate with the percentage decrease in forced expiratory volume in 1 second or with the change in large airway resistance (R20), but it did correlate with the percentage decline in maximal expiratory flow at 50% vital capacity and with the change in small airway resistance (R5-R20). The eNO decrease lasted for 15 minutes after β_2 -agonist inhalation in the group with a high percentage decrease in R5-R20 ($>200\%$). On the other hand, in the group with a low percentage decrease in R5-R20 ($\leq 200\%$), eNO recovered to the previous level immediately after β_2 -agonist inhalation.

Conclusions: The eNO level significantly decreases after methacholine inhalation challenge. This decrease primarily depends on bronchoconstriction of the small airways.

Ann Allergy Asthma Immunol. 2009;102:469–474.

INTRODUCTION

The measurement of exhaled nitric oxide (eNO) has been proposed as a noninvasive marker of airway inflammation, especially in asthma.^{1–4} Levels of eNO are useful for diagnosing childhood asthma and for predicting asthma relapse in children with clinical asthma remission.^{5–7} Recent studies have shown that levels of eNO are decreased by anti-inflammatory treatment,⁸ and eNO has been proposed as a means of monitoring the efficacy of treatment in asthmatic patients.³

Despite the many studies of eNO in asthma, the relationship between bronchoconstriction and eNO levels is still unclear. Previous studies⁹ have demonstrated a reduction in the eNO level immediately after methacholine challenge that does not correlate with the percentage decrease in forced expiratory volume in 1 second (FEV₁). Several studies^{10,11} have also shown that eNO values increase after β_2 -agonist therapy, but its relationship to bronchodilation remains unclear.

We aimed to evaluate whether the eNO level is affected by bronchoconstriction in healthy and asthmatic children. Furthermore, respiratory resistance (Rrs) was evaluated sepa-

rately in the large and small airways to determine the effect of airway size on changes in eNO levels.

METHODS

Participants

Ninety-two children aged 5 to 15 years were evaluated at Gunma University Graduate School of Medicine. Sixty-two children with asthma, 13 wheezy children, and 17 healthy children were enrolled. To determine the patients with asthma, the parents of each child completed an American Thoracic Society/Division of Lung Diseases respiratory questionnaire. If the child had more than 3 lifetime episodes of wheezing and was diagnosed as having asthma by a physician, he or she was determined to have asthma. Wheezy children received diagnoses when they had fewer than 3 lifetime episodes of wheezing and were not diagnosed as having asthma by a physician. Healthy children were those without asthma or other respiratory diseases. We performed lung function tests on healthy children who visited Gunma University Graduate School of Medicine independently for the health checkup just before entering elementary school. Because healthy children also showed bronchoconstriction after methacholine challenge, we involved the data in this study. Approval was obtained from the hospital ethics committee, and informed parental consent for each child was obtained before the study.

Affiliations: * Department of Pediatrics and Developmental Medicine, Gunma University Graduate School of Medicine, Gunma, Japan; † Department of Pediatrics, Yokohama City University School of Medicine, Kanagawa, Japan.

Disclosures: Authors have nothing to disclose.

Received for publication October 27, 2008; Received in revised form January 7, 2009; Accepted for publication January 29, 2009.

Study Design

Spirometry, airway resistance, and eNO measurements were performed before and after methacholine provocation and immediately and 15 minutes after β_2 -agonist (salbutamol) inhalation (Fig 1). At each point of measurement, eNO levels were measured before lung function testing and measurement of Rrs. Every participant inhaled salbutamol hemisulfate (1.7 mg/mL in saline solution) for 2 minutes or more until Rrs recovered to the previous level. Previous data¹² suggest that the total amount of nebulized salbutamol sufficient to induce bronchodilation against methacholine-induced bronchoconstriction was approximately 0.5 mg.

eNO Measurement

Online measurement was performed according to European Respiratory Society/American Thoracic Society recommendations⁶ using a chemiluminescence analyzer (model 280i nitric oxide analyzer; Sievers, Boulder, Colorado). Daily calibration was performed using 100% nitrogen to zero and an analyzed standard gas (NO, 0.64 ppm) (Taiyonsan, Tokyo, Japan) for the span. Patients exhaled from total lung capacity to residual volume without holding their breath at a constant flow rate of 50 mL/s. The environmental NO level was measured before each study, and samples measured when the environmental NO level exceeded 10 ppb were excluded. All the participants were measured in the sitting position without wearing a nose clip.

Methacholine Inhalation Challenge

Methacholine inhalation challenges were performed according to the procedure described by Takishima et al.¹ An Astograph aerosol generator (TCK 6100H; Chest Co, Tokyo) delivered increasing doses of methacholine from 12 serially arranged nebulizers. The nebulizers were activated by a constant switch from 1 nebulizer to the next at predetermined intervals. Each nebulizer delivered approximately 0.15 mL of solution per minute of increasing concentrations of methacholine and bronchodilator.

Bronchial responsiveness was displayed in the form of a continuous record of Rrs, as measured using the oscillation technique. A constant-amplitude pressure generator was con-

nected to a mouthpiece that produced a constant-amplitude sinusoidal pressure wave of 2 cm H₂O of 7 Hz at the mouth. Recordings showing excessive fluctuations, caused by coughing, were excluded after 2 independent examiners agreed that such records were not suitable for use in the evaluation of bronchial hyperresponsiveness.

Methacholine (Daiichi Kagaku Yakuhin, Tokyo), prepared on the day of the test, was serially diluted 2-fold with saline solution (10 dose steps starting at 25 mg/mL down to approximately 49 μ g/mL). Nebulizer 1 contained 2 mL of saline solution, nebulizer 2 contained 2 mL of the highest dilution of methacholine, and nebulizers 3 to 11 contained increasing concentrations of methacholine. Each concentration of the methacholine solution was inhaled for 1 minute. Nebulizer 12 contained salbutamol hemisulfate (1.7 mg/mL in saline solution) and was inhaled for 2 minutes.

Patients were examined during quiet breathing in a sitting position with a nose clip attached and 2 air-filled balloons pressing against both cheeks; doses of inhaled methacholine were doubled every minute. When the Rrs reached double the baseline value, methacholine administration was stopped and salbutamol solution was administered. The total amount of nebulized salbutamol was approximately 0.5 mg. The Rrs was continuously measured until it reached a stable state.

Impulse Oscillometry System

Airway reactance in response to methacholine inhalation challenge was determined using an impulse oscillometry system (Jaeger MS-IOS and LAB Manager Software version 4.53.2, 2002; Erich Jaeger, Hoechst, Germany) using the technique recommended by the manufacturer. The children used nose clips, and the guardian gently held the sides of each child's face to decrease the shunt compliance of the cheeks. Real-time recordings of mouth pressure and flow signals pulsed through a 5- to 20-Hz spectrum were superimposed over tracings of tidal breathing and were displayed on a computer screen. Measurements of resistance at 5 and 20 Hz (R₅, R₂₀, and R₅-R₂₀), reactance at 5 Hz, and the low-frequency reactance area (the area of reactance integrated from 5 Hz up to resonant frequency) were recorded. It was agreed that a value of R₂₀ demonstrated central Rrs and a value of R₅-R₂₀ demonstrated peripheral Rrs.¹³⁻¹⁵

Spirometry

Pulmonary function was determined by means of spirometry using a calibrated computerized pneumotachograph spirometer (MICROSPIRO HI-601; Chest Co). Standard measures of pulmonary function were collected, including forced vital capacity, FEV₁, maximal expiratory flow at 50% vital capacity (V'₅₀), and maximal expiratory flow at 25% vital capacity per height. The procedure for all pulmonary function tests (PFTs) included the following: (1) 3 normal tidal volume breaths followed by (2) inhalation to total lung capacity and (3) forced maximal exhalation

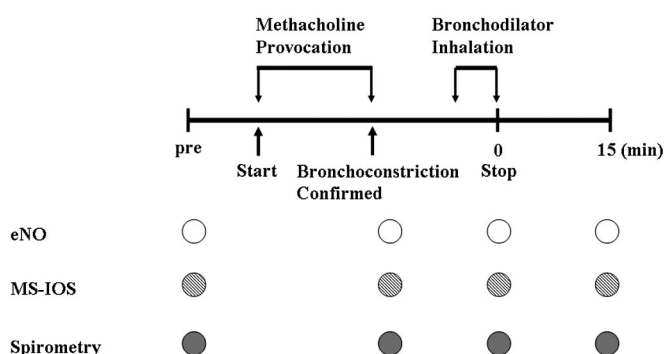


Figure 1. Measurement protocol. eNO indicates exhaled nitric oxide; MS-IOS, impulse oscillometry system.

terminating with a final maximal inhalation. All tests were performed according to current American Thoracic Society spirometry standards. The resting baseline was determined by selecting the best of 3 resting PFT results based on the highest sum of the forced vital capacity and FEV₁. One maneuver was performed at each postchallenge time point in an effort to minimize participant fatigue and to guard against the potential effects of deep inhalation. However, if any baseline or postchallenge time point measurement was technically unacceptable, then the PFT maneuver was repeated immediately.

Statistical Analysis

Statistical analyses were performed using a computer software package (SPSS version 11.0; SPSS Inc, Chicago, Illinois). Comparisons of each of the variables in healthy, wheezy, and asthmatic participants were made using the Mann-Whitney test. Differences in eNO levels before and after methacholine testing and salbutamol inhalation were analyzed using the Wilcoxon test. Spearman correlation coefficients were calculated to evaluate the statistical association between percentage change in spirometry values and eNO levels. Correlations among the percentage change in airway resistance and eNO levels were made using the Pearson correlation test. Data are expressed as mean (SEM). $P < .05$ was considered statistically significant.

RESULTS

Spirometry, Airway Resistance, and Methacholine Testing

The anthropometric, lung function, and eNO data are given in Table 1. The percentage change in FEV₁ and V'50 significantly decreased after methacholine challenge and recovered 15 minutes after salbutamol inhalation (Fig 2). The percentage change in the R5-R20 after methacholine challenge was higher than that in the R20 (250% vs 16%) (Fig 3). Although the percentage change in R20 recovered immediately after salbutamol inhalation, the percentage change in R5-R20, the low-frequency reactance area, and reactance at 5 Hz

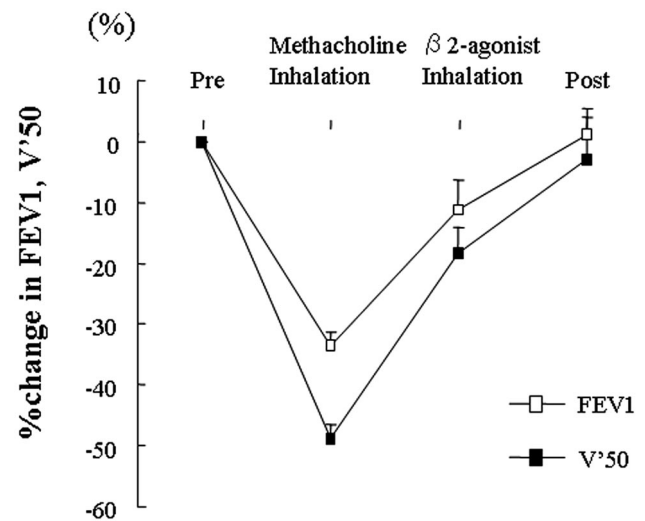


Figure 2. Percentage change in forced expiratory volume in 1 second (FEV₁) and maximal expiratory flow at 50% vital capacity (V'50) after methacholine challenge and nebulized salbutamol infusion. Error bars represent SEM.

did not recover until 15 minutes after salbutamol inhalation (Fig 3).

eNO Level and Methacholine Testing

A significant decrease was seen in the eNO level after methacholine inhalation challenge ($P < .01$) (Fig 4A). Although the eNO level decrease lasted 15 minutes after salbutamol inhalation in the group with a high percentage change in the R5-R20 ($>200\%$), the eNO level recovered immediately after inhalation in the group with a low percentage change in the R5-R20 ($\leq 200\%$) (Fig 4B). The decrease in the eNO level did not significantly correlate with the percentage change in FEV₁ ($r = 0.184$, $P = .11$) or with the difference in R20 ($r = 0.157$, $P = .18$) but did correlate with the percentage change in V'50 ($r = 0.240$, $P = .04$) and with the

Table 1. Anthropometric, Lung Function, and eNO Data^a

	Participants			P value ^b
	Healthy (n = 17)	Wheezy (n = 13)	Asthmatic (n = 62)	
Sex, M/F, No.	11/6	9/4	42/20	
Age, y	5.8 (0.39)	6.6 (2.2)	10.0 (3.3)	<.001
Height, m	1.15 (0.06)	1.16 (0.11)	1.39 (0.19)	<.001
Weight, kg	22.4 (4.1)	22.1 (5.0)	38 (15.6)	<.001
FVC, %	86.7 (11.4)	86.9 (12.1)	96.4 (15.9)	NS
FEV ₁ , %	108.2 (3.1)	100.8 (19.2)	98.7 (9.8)	<.001
eNO, ppb	21.8 (7.6)	24.0 (18.5)	60.6 (48.8)	<.01

Abbreviations: eNO, exhaled nitric oxide; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; NS, not significant.

^a Data are expressed as mean (SD) except where indicated otherwise.

^b The P value indicates the statistical significance of differences between healthy and asthmatic participants.

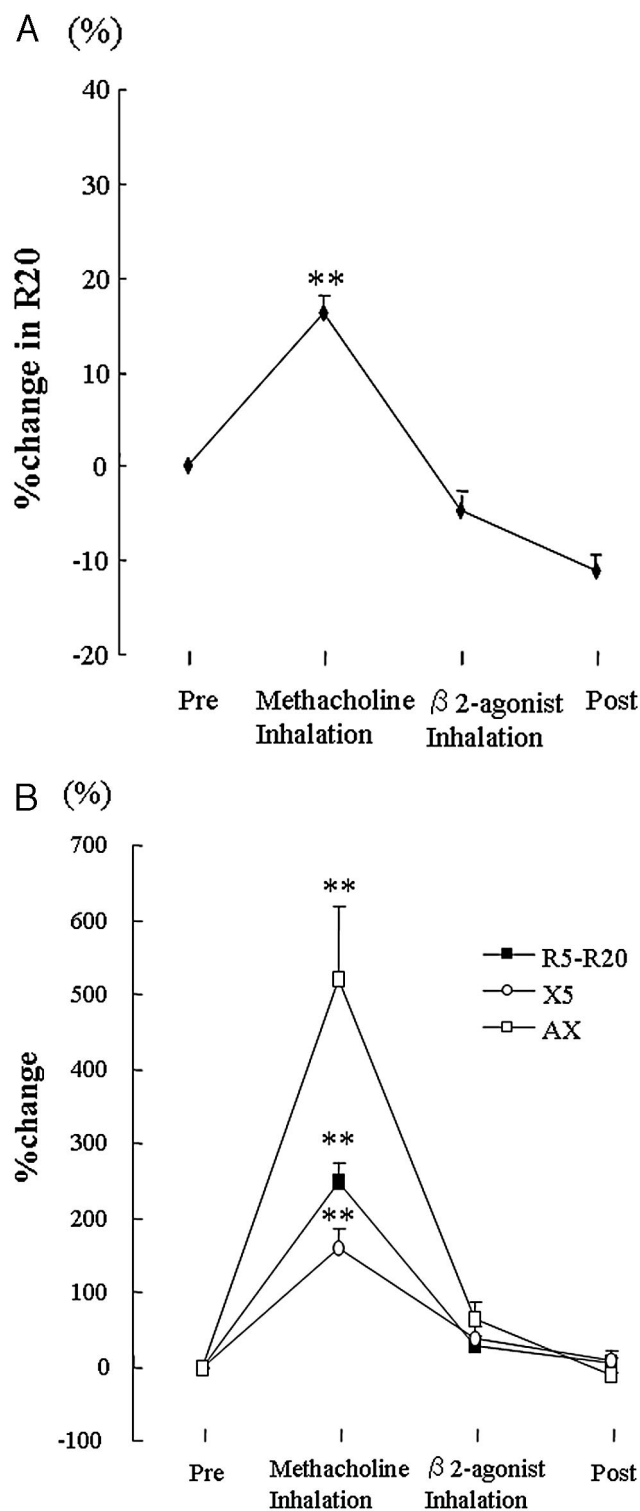


Figure 3. Percentage change in large (R20) (A) and small (R5-R20) (B) airway resistance after methacholine challenge and nebulized salbutamol infusion. Error bars represent SEM. ** $P < .01$. AX indicates low-frequency reactance area; X5, reactance at 5 Hz.

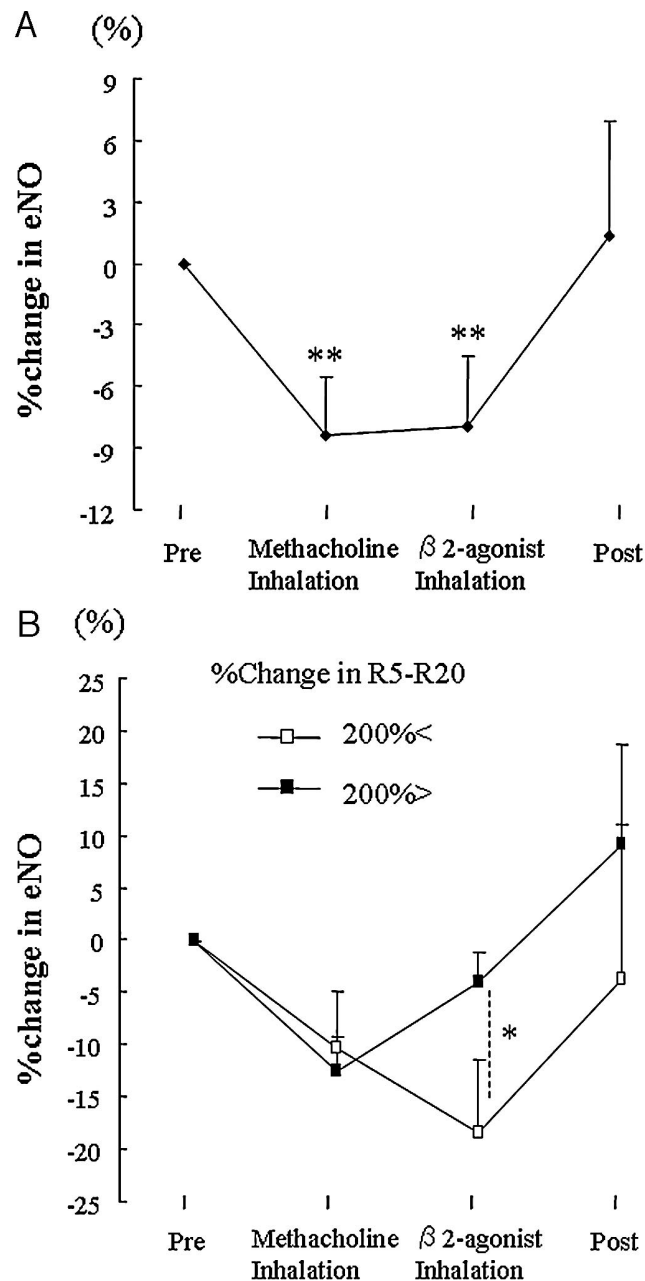


Figure 4. Percentage change in exhaled nitric oxide (eNO) levels after methacholine challenge and nebulized salbutamol infusion. A, Percentage change in eNO levels in all patients. B, Effect of small airway (R5-R20) on the percentage change in eNO levels. Error bars represent SEM. * $P < .05$ between the 2 groups. ** $P < .01$.

difference in the R5-R20 ($r = 0.269$, $P = .02$) (data not shown).

DISCUSSION

These results show that acute bronchoconstriction of the small airway leads to a rapid decrease in eNO levels in

children. This is the first study demonstrating the effect of acute bronchoconstriction of the small airways on the eNO level using spirometry and airway resistance.

Although previous studies have shown that eNO levels decrease after methacholine bronchial provocation,^{9,16} the mechanism of action remains to be elucidated. de Gouw et al¹⁷ observed a decrease in the eNO level after bronchial provocation with histamine and adenosine monophosphate that was significantly correlated with the percentage decrease in FEV₁. The authors concluded that the decrease in the eNO level was not caused by the procedure itself but was related to the decrease in FEV₁, suggesting that eNO levels are modulated by the degree of airway obstruction. On the other hand, Piacentini et al⁹ observed a decrease in the eNO level after bronchial provocation with methacholine that was not significantly correlated with the decrease in FEV₁. Furthermore, Verbanck et al¹⁸ observed a significant correlation of eNO decrease with forced expiratory flow between 25% and 75% decrease but not with FEV₁ decrease or with increased ventilation heterogeneity. The present results support these earlier findings because the decrease in the eNO level did not correlate with the decrease in FEV₁ and significantly correlated with the decrease in V₅₀, which is one of the variables of the small airways.

The impulse oscillometry system was introduced as a new technique for the evaluation of airway obstruction in patients who cannot perform forced breathing maneuvers, such as young children.^{19,20} Vink et al²¹ demonstrated that the resistance and reactance values showed significant changes during changes in airflow obstruction by means of methacholine challenge. In the present study, the decrease in the eNO level after methacholine challenge did not correlate with the increase in large airway resistance (R20) but was correlated with the increase in small airway resistance (R5-R20). Furthermore, when bronchoconstriction of the small airway was tight, the eNO level did not return to the baseline level immediately after bronchodilator inhalation and lasted until 15 minutes later.

These data suggest that eNO levels are modulated by the degree of bronchoconstriction of the small airways. Because NO originates predominantly from the terminal and respiratory bronchioles,²² these findings might be due to the relative reduction in the ventilated airway surface during bronchoconstriction of the small airways. Constriction of the small airways, thus causing a reduction of the luminal airway surface,^{23,24} may, therefore, lead to decreased eNO levels in exhaled air. In addition, it can be speculated that trapping of NO in the small airways during bronchoconstriction contributes to the presently observed reductions in eNO levels.

In conclusion, the present results show that eNO levels significantly decrease after methacholine inhalation challenge, primarily due to bronchoconstriction of the small airways. In the measurement of eNO, the effect of bronchoconstriction of the small airways should, therefore, be carefully taken into consideration.

ACKNOWLEDGMENTS

We thank Drs Tamotsu Ishizuka and Masatomo Mori, Department of Medicine and Molecular Science, Gunma University Graduate School of Medicine, for their technical assistance.

REFERENCES

1. Baraldi E, Jongste JC, Gaston B, et al. Measurement of exhaled nitric oxide in children, 2001. *Eur Respir J*. 2002;20:223–227.
2. American Thoracic Society, European Respiratory Society. ATS/ERS recommendations 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.
3. Byrnes CA, Dinarevic S, Shinebourne EA, et al. Exhaled nitric oxide measurements in normal and asthmatic children. *Pediatr Pulmonol*. 1997;24:312–318.
4. Saito J, Inoue K, Sugawara A, et al. Exhaled nitric oxide as a marker of airway inflammation for an epidemiologic study in school children. *J Allergy Clin Immunol*. 2004;114:512–516.
5. 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.
6. Fitzpatrick AM, Gaston BM, Erzurum SC, et al. Features of severe asthma in school-age children: atopy and increased exhaled nitric oxide. *J Allergy Clin Immunol*. 2006;118:1218–1225.
7. Pijnenburg MW, Hofhuis W, Hop WC, et al. Exhaled nitric oxide predicts asthma relapse in children with clinical asthma remission. *Thorax*. 2005;60:215–218.
8. Kharitonov SA, Yates DH, Barnes PJ. Inhaled glucocorticoids decrease nitric oxide in exhaled air of asthmatic patients. *Am J Respir Crit Care Med*. 1996;153:454–457.
9. Piacentini GL, Bodini A, Peroni DG, et al. Reduction in exhaled nitric oxide immediately after methacholine challenge in asthmatic children. *Thorax*. 2002;57:771–773.
10. Kisson N, Duckworth LJ, Blake KV, et al. Effect of β_2 -agonist treatment and spirometry on exhaled nitric oxide in healthy children and children with asthma. *Pediatr Pulmonol*. 2002;34:203–208.
11. Silkoff PE, Wakita S, Chatkin J, et al. Exhaled nitric oxide after β_2 -agonist inhalation and spirometry in asthma. *Am J Respir Crit Care Med*. 1999;159:940–944.
12. Mochizuki H, Shigeta M, Kato M, et al. Age-related changes in bronchial hyperreactivity to methacholine in asthmatic children. *Am J Respir Crit Care Med*. 1995;152:906–910.
13. Goldman MD, Carter R, Klein R, et al. Within- and between-day variability of respiratory impedance, using impulse oscillometry in adolescent asthmatics. *Pediatr Pulmonol*. 2002;34:312–319.
14. Mansur AH, Manney S, Ayres JG. Methacholine-induced asthma symptoms correlate with impulse oscillometry but not spirometry. *Respir Med*. 2008;102:42–49.
15. Tanaka H, Fujii M, Tanaka Y, Tanaka N, Takahashi H. Contribution of small airway resistance in adult severe persistent asthmatic patients assessed by the impulse oscillation system. *Respir Res*. 2007;26: 675–679.
16. Ho CF, Wang CH, Liu CY, et al. The effect of bronchodilators on exhaled nitric oxide (NO) in patients with bronchial asthma. *Eur Respir J*. 1997;10:1025.
17. de Gouw HWFM, Hendriks J, Woltman AM, et al. Exhaled nitric oxide (NO) is reduced shortly after bronchoconstriction to direct and indirect stimuli in asthma. *Am J Respir Crit Care Med*. 1998;158: 315–319.
18. Verbanck S, Kerckx Y, Schuermans D, Vincken W, Paiva M, Muylem AV. Effect of airways constriction on exhaled nitric oxide. *J Appl Physiol*. 2008;104:925–930.
19. Vogel J, Schmidt U. *Impulse Oscillometry Analysis of Lung Mechanics in General Practice and the Clinic: Epidemiologic and Experimental Research*. Frankfurt, Germany: PMI Verlagsgruppe; 1994.

-
20. Hellinckx J, Cauberghs M, De Boeck K, et al. Evaluation of impulse oscillation system: comparison with forced oscillation technique and body plethysmography. *Eur Respir J*. 2001;18:564–570.
 21. Vink GR, Arets HGM, van der Laag J, et al. Impulse oscillometry: a measure for airway obstruction. *Pediatr Pulmonol*. 2003;35:214–219.
 22. Persson MG, Wiklund NP, Gustafsson LE. Endogenous nitric oxide in single exhalations and the change during exercise. *Am Rev Respir Dis*. 1993;148:1210–1214.
 23. James AL, Pare PD, Hogg JC. Effects of lung volume, bronchoconstriction, and cigarette smoke on morphometric airway dimensions. *J Appl Physiol*. 1988;64:913–919.
 24. Yager D, Bulter JP, Bastacky J, et al. Amplification of airway constric-

tion due to liquid filling of airway interstices. *J Appl Physiol*. 1989;66:2873–2884.

Requests for reprints should be addressed to:
Hiromi Tadaki, MD
Department of Pediatrics
Yokohama City University School of Medicine
3-9 Fukuura
Kanazawaku
Yokohama City
Kanagawa 236-0004, Japan
E-mail: hiromic@pop06.odn.ne.jp
