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Reduced Exhaled Nitric Oxide in Children After Testing of Maximal Expiratory Pressures

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Summary. Spirometry in adult subjects can induce a fall in concentration of exhaled nitric oxide (FE_{NO}). Scarce information is available on the FE_{NO} decrease after spirometry or after other forced lung-function maneuvers in children. We compared changes in FE_{NO} induced by repeated spirometry and testing of maximal expiratory pressures (P_{Emax}). Twenty-four sex- and age-matched children aged 9–18 years (mean age ± SD, 13.3 ± 2.8 years; 12 healthy, 12 asthmatic) were allocated to 1-week-apart sessions of repeated maneuvers of either forced vital capacity (FVC) or P_{Emax}. Baseline FE_{NO} measurements were followed by FVC or P_{Emax} maneuvers every 15 min for 45 min, whereas FE_{NO} was measured at each step for 60 min. After repeated P_{Emax} but not after FVC maneuvers, FE_{NO} values decreased significantly from baseline in both groups. In healthy children, geometric mean FE_{NO} (95% confidence intervals) decreased from 9.1 (7.0–11.8) ppb at baseline to 8.2 (6.3–10.6) ppb at 15 min and 7.7 (5.6–10.6) ppb at 30 min ($P < 0.05$ and $P < 0.01$, respectively), and remained unchanged at 45 and 60 min. In asthmatic children, FE_{NO} levels fell from 21.6 (13.3–34.9) ppb at baseline to 15.1 (9.1–25.1) ppb at 15 min and remained low at 30, 45, and 60 min: 17.8 (10.7–29.5) ppb, 17.5 (10.2–30.1) ppb, and 17.6 (10.6–29.2) ppb, $P < 0.01$, for all differences from baseline. Repeated P_{Emax} and FVC maneuvers increased FE_{NO} variability, as compared with repeated FE_{NO} measurements alone. Previous forced lung-function maneuvers may affect FE_{NO} measurements in children. Although P_{Emax} testing has a greater influence than spirometry on FE_{NO} levels in children, both procedures should be avoided before measuring FE_{NO}. **Pediatr Pulmonol. 2006; 41:141–145.** © 2005 Wiley-Liss, Inc.

Key words: asthma; lung-function testing; exhaled nitric oxide.

INTRODUCTION

The most widely used noninvasive marker of airway inflammation, especially in asthma, is the fractional concentration of exhaled nitric oxide (FE_{NO}). FE_{NO} measurements and lung-function tests are usually obtained in a single session. Because FE_{NO} levels could be affected by taxing respiratory maneuvers, the American Thoracic Society (ATS)/ European Respiratory Society (ERS) guidelines recommend avoiding them before FE_{NO} measurements.[1,2] Several studies investigated the effects of spirometry on FE_{NO} in adults, and the majority showed that FE_{NO} decreases.[3–5] Only two studies addressed this issue in children, but with contrasting results.[6,7] Current knowledge on the effects of respiratory tests on FE_{NO} is limited to the FVC maneuver, but FE_{NO} levels might also be influenced by other maneuvers, such as maximal expiratory pressures (P_{Emax}). The practical importance of these matters lies in saving time in the outpatient departments, and sparing children unnecessarily lengthy testing sessions.

In this study, we investigated changes in FE_{NO} induced by lung-function testing in children. We measured FE_{NO} before and after repeated maneuvers for spirometry (forced vital capacity, FVC) and P_{Emax} in healthy and asthmatic children. Some of the results of this study were previously reported in abstract form.[8]

METHODS

Subjects

We recruited 12 healthy and 12 asthmatic children matched for age and sex (aged 9–18 years; 12 healthy, 12 asthmatic; M/F, 6/6 by group). Healthy participants were drawn from children attending an ambulatory-routine health control. They were nonatopic, had no history of chronic respiratory symptoms, and had normal spirometry and bronchial responses to inhaled albuterol.

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Asthmatic children were diagnosed in accordance with ATS guidelines.[9] They had mild-intermittent asthma, with forced expiratory volume in 1 sec (FEV_1) values over 80% predicted at recruitment, and had previously documented bronchial hyperresponsiveness, as measured by either the bronchial response to albuterol (FEV_1 increase, >12% of predicted) or a positive response to exercise testing (FEV_1 fall over 12% of baseline). None of them had taken inhaled or oral steroids in the previous 2 weeks, and needed only rescue medication with inhaled β-agonists. No subject had acute respiratory illness for at least 4 weeks before testing. Informed consent was obtained from parents, and the study was approved by the hospital Ethics Committee.

Study Design

Each subject was assigned in random order to two protocols, one protocol per session 1 week apart, comprising repeated FVC (spirometry session) or maximal expiratory pressure (P_{Emax} session) maneuvers. FVC and P_{Emax} maneuvers were performed at baseline, and testing was repeated every 15 min for 45 min. FE_{NO} was measured at baseline before lung-function testing, and repeated in the same order every 15 min for 1 hr. To achieve comparability with similar studies in adults, the intervals elapsing between FE_{NO} and lung-function measurements were the same as in a previously described design.[4] These intervals were selected to assess whether lung function-induced changes in FE_{NO} persisted after 15 min, and to assess further FE_{NO} decay after repeated maneuvers. All sessions took place in the morning, starting at 9.00 AM. From the 24 recruits, two subgroups (6 healthy children and 6 asthmatic children) were drawn, and each subject underwent repeated FE_{NO} measurements in a separate session (every 15 min for 1 hr) without previous spirometry or P_{Emax} maneuvers (control session).

Lung-Function Measurements

Spirometry

FVC maneuvers were recorded with a portable spirometer (heated Fleisch pneumotachograph ESS-γ, Biomedin s.r.l., Padua, Italy), with subjects in standing position. Duplicate measurements from at least three acceptable maneuvers were obtained and recorded as recommended.[10] The average number of FVC maneuvers (±SD), whether acceptable or not, performed after each FE_{NO} measure (baseline, and 15, 30, 45, and 60 min), was 3.9 ± 0.8, and remained unchanged at all times. Values were expressed as percent predicted.[11]

P_{Emax} Measurements

Duplicate sets of P_{Emax} values were obtained with a portable device (Morgan Pmax, Morgan Italia s.r.l., Lazzaro di Savena, Bologna, Italy). Subjects were tested in the seated position without noseclips, wearing tube-style mouthpieces with a small leak.[12–14] Procedures followed the ATS/ERS statement.[12] The average number of P_{Emax} maneuvers (±SD), whether acceptable or not, performed after each FE_{NO} measure (baseline, and 15, 30, 45, and 60 min), was 3.0 ± 0.8, and remained unchanged at all times.

Values were expressed in cm H_2O and as percent predicted.[15]

FE_{NO} Measurements

FE_{NO} was measured by chemiluminescence (Sievers NOA™ 280, Sievers Instruments, Inc., Boulder, CO), as previously reported.[16] The single-breath online technique with inhalation of NO-free air was used.[17] All participants were asked to avoid food intake and physical exercise for at least 2 hr before testing. Subjects did single-breath maneuvers in duplicate at a constant expiratory pressure and flow (10 cm H_2O and 50 ml/sec),[2] as described in detail elsewhere.[16]

Low ambient averaged NO levels were recorded during the sessions (range, 1.5–9 ppb).

Statistical Analysis

FE_{NO} values were non-normally distributed (Kolmogorov-Smirnov test); they were transformed to natural logarithms (Ln), and expressed as geometric means and 95% confidence intervals (CI). Differences between geometric means were expressed as mean ratios and 95% CI. Lung-function variables were normally distributed and expressed as arithmetic means and 95% CI. The Wilcoxon signed-ranks test was used to compare baseline measurements between sessions. Nonparametric analysis of variance (ANOVA; Friedman test) with the post hoc Dunnett test was used to assess significant differences between measurements obtained at each step against measurements at baseline. The within-subject coefficient of variation (CV = SD/mean) was used to assess repeatability of FE_{NO} measurements. Statistical software (SPSS 10, SPSS, Inc., Chicago, IL) was used for calculations. *P*-values less than 0.05 were considered statistically significant.

RESULTS

Among the 12 healthy children, no difference was found between baseline FE_{NO} for the spirometry session

ABBREVIATIONS

FE_{NO}	Exhaled nitric oxide
FEV_1	Forced expiratory volume in 1 sec
FVC	Forced vital capacity
P_{Emax}	Maximal expiratory pressures

and the P_{Emax} session (geometric means, 8.0 vs. 9.1 ppb; mean ratio, 0.88; 95% CI, 0.68–1.14). In the subgroup of 6 healthy children who underwent the control session, the baseline FE_{NO} geometric mean (95% CI) was 8.2 (4.4–11.9) ppb. Among the 12 asthmatic children, baseline FE_{NO} was significantly lower for the spirometry session than for the P_{Emax} session (geometric means, 16.5 vs. 21.6 ppb; mean ratio, 0.76; 95% CI, 0.65–0.89; $P < 0.01$). In the subgroup of 6 asthmatic children who underwent the control session, the baseline FE_{NO} geometric mean (95% CI) was 18.8 (9.9–27.6) ppb (Table 1).

In healthy and asthmatic subjects, the spirometry session and repeated FE_{NO} measurements alone (control session) left FE_{NO} levels unchanged. In contrast, in both groups, after P_{Emax} maneuvers, FE_{NO} decreased for at least 30 min. P_{Emax} and FVC measurements were similar in the two groups of children, and remained unchanged throughout the experiment (Table 1).

The initial fall in FE_{NO} with P_{Emax} maneuvers was statistically significant, both in the 6 asthmatic subjects in whom baseline FE_{NO} values did not differ between P_{Emax} and spirometric sessions, and in those in whom they did. On the other hand, the time dependency of FE_{NO} changes after P_{Emax} maneuvers differed between asthmatic children with high or low baseline FE_{NO} values. In those with low values, as in healthy children, FE_{NO} returned to baseline values at 45 and 60 min (Table 2).

Mean CV values for repeated FE_{NO} measurements alone (control session) were 4.3% in healthy subjects and 3.9% in asthmatic subjects. Within-subject variability increased, however, both after repeated P_{Emax} maneuvers (CV, 10.5% in healthy and 16.9% in asthmatic children) and after FVC maneuvers (CV, 11.1% and 7.4%).

DISCUSSION

Our main finding was that in healthy children and in those with asthma, repeated P_{Emax} maneuvers consistently decreased subsequent FE_{NO} measurements. Although repeated FVC maneuvers and repeated FE_{NO} measurements alone (without P_{Emax} or FVC maneuvers) left subsequent FE_{NO} values unchanged, repeated FVC maneuvers induced high FE_{NO} variability (about twice the CV obtained with repeated FE_{NO} measurements alone). Hence, to save time and avoid keeping children waiting more than 15 min between maneuvers, FE_{NO} should be measured before P_{Emax} and spirometry.

In a previous study, we found that in one group of healthy and asthmatic children, repeated P_{Emax} maneuvers decreased FE_{NO}, whereas in another group, spirometric

TABLE 1—Effects of the Various Protocols on Fe_{NO} in Healthy and Asthmatic Children[1]

		Baseline	15 min	30 min	45 min	60 min
Spirometry session						
FEV_1, % predicted	Healthy	104.7 (98.6–110.9)	104.4 (97.9–110.8)	103.3 (97.5–109.2)	104.2 (98.7–109.7)	
	Asthmatic	101.0 (92.6–109.4)	99.9 (91.4–108.5)	100.3 (91.7–109.0)	100.9 (92.3–109.4)	
FE_{NO}, ppb	Healthy	8.0 (6.8–9.4)	8.8 (7.2–10.8)	8.5 (7.3–9.8)	8.9 (7.7–10.3)	8.9 (7.5–10.4)
	Asthmatic	16.5 (10.3–26.4)	17.1 (10.7–27.5)	17.5 (11.1–27.5)	17.2 (10.8–27.5)	16.9 (10.6–26.9)
P_{Emax} session						
P_{Emax}, cmH_2O	Healthy	110.9 (94.4–127.4)	113.6 (94.7–134.1)	114.1 (99.8–134.1)	120.3 (99.8–140.8)	
	Asthmatic	104.7 (90.5–118.9)	103.6 (87.1–120.2)	103.3 (87.3–119.3)	101.6 (85.8–117.4)	
FE_{NO}, ppb	Healthy	9.1 (7.0–11.8)	8.2* (6.3–10.6)	7.7** (5.6–10.6)	8.3 (6.1–11.2)	8.9 (6.9–11.5)
	Asthmatic	21.6 (13.3–34.9)	15.1** (9.1–25.1)	17.8** (10.2–30.1)	17.5** (10.2–30.1)	17.6** (10.6–29.2)
Control session						
FE_{NO}, ppb	Healthy	8.2 (4.4–11.9)	8.1 (4.5–11.7)	8.3 (4.1–12.5)	8.5 (4.8–12.2)	8.5 (4.5–12.4)
	Asthmatic	18.8 (9.9–27.6)	18.5 (9.6–27.4)	18.5 (9.8–27.2)	18.2 (9.3–27.3)	19.3 (9.0–29.6)

[1]Values are arithmetic (FEV_1) or geometric means (FE_{NO}) and 95% confidence intervals. Number of subjects: for spirometry and P_{Emax} sessions, 12 healthy and 12 asthmatics; for control session, 6 healthy and 6 asthmatic.
*$P < 0.05$, compared with baseline values.
**$P < 0.01$, compared with baseline values.

TABLE 2— Effect of Repeated P_{Emax} Maneuvers on FE_{NO} in Asthmatic Children With Low and High Baseline Values[1]

Baseline	15 min	30 min	45 min	60 min
16.8 (7.1–39.8)	10.9** (5.1–23.3)	14.9* (6.2–35.5)	14.7 (5.7–38.1)	15.6 (6.4–38.0)
27.6 (13.6–55.9)	20.8** (8.9–48.6)	21.3* (9.2–49.1)	20.9** (8.9–49.7)	19.7** (8.4–46.2)

[1]Values (ppb) are geometric means (FE_{NO}) and 95% confidence intervals.
*$P < 0.05$, compared with baseline values.
**$P < 0.01$, compared with baseline values.

maneuvers did not.[8] The present results confirm these findings in subjects who underwent both procedures (repeated P_{Emax} and FVC). Moreover, they suggest that the fall in FE_{NO} is not appreciably potentiated by additional lung-function maneuvers, but attenuates with time and eventually vanishes.

Although baseline FE_{NO} levels in asthmatic children were significantly higher at the P_{Emax} session than during the spirometry session, the P_{Emax}-induced FE_{NO} decrease remained statistically significant in the 6 asthmatic subjects whose baseline FE_{NO} remained comparably low at both sessions. The unexpected increase in baseline FE_{NO} of asthmatic children during the P_{Emax} session cannot be attributed to ambient NO contamination, because ambient NO levels were consistently low throughout the study. Nor can they be attributed to high FE_{NO} variability in asthmatic children.[18,19] We cannot exclude the possibility that before the P_{Emax} session, some of our asthmatic children could have been challenged by allergens to which they were previously sensitized.

Our results in children contrast with previous findings suggesting that repeated spirometry reduces FE_{NO} in adults,[3–5] an effect attributed to airway closure[5] or to inhibition of nonadrenergic noncholinergic nerves within the airways.[3] The discrepant results cannot be explained by differences in design, because the interval we used between FE_{NO} and spirometry was the same as that used by Deykin et al.[4] The FE_{NO} decay observed by Silkoff et al. started the first minute after FVC maneuvers, and persisted for 45 min or longer.[5] In line with others,[20] our group failed to find a similar decay in young adults (unpublished data).

In children, one study reported that FE_{NO} tended to decrease after 5 and 15 min of FVC maneuvers.[7] In contrast, in small groups of healthy and asthmatic children, Kissoon et al. found that FE_{NO} tended to increase after 3, 8, and 18 min of FVC maneuvers.[6] Our data in children and our unpublished data in adults suggest that spirometry influences FE_{NO} in a similar way in adults and children.

Why repeated P_{Emax} (Valsalva maneuver), but not repeated spirometry, decreased FE_{NO} is difficult to explain. Both maneuvers can induce rapid changes in systemic arterial pressure, heart rate, vagal and sympathetic efferent discharges, and pleural pressure.[21] On the other hand, P_{Emax} maneuvers involve higher and more sustained pleural pressure than do FVC maneuvers. During FVC maneuvers, pressures at every volume are reduced from maximal static pressures, because expiratory muscles are shortening.[12] A higher epithelial surface area could therefore be stressed more during P_{Emax} than during FVC maneuvers, and such high stresses might decrease NO production. Moreover, during repeated P_{Emax} maneuvers each lasting 3–4 sec, the airways are presumably exposed to high NO concentrations, as they are during breath-holding, and this might reduce NO production from airway epithelium.

We consider it highly unlikely that the reduced FE_{NO} levels we describe arose from autonomic responses elicited by Valsalva maneuvers performed at total lung capacity. The effects induced by changing hemodynamics occur rapidly and rapidly vanish, whereas we measured FE_{NO} 15 min after P_{Emax} maneuvers. Accordingly, in our unpublished study in adults, we found no changes in heart rate and arterial pressure 5 min after spirometric maneuvers.

In this study, we measured FE_{NO} at an expiratory flow of 50 ml/sec, the recommended flow for assessing children.[1,2] At this flow, FE_{NO} mainly reflects NO concentrations diffused into the airway lumen, but also includes a small amount of alveolar NO carried by convection.[22] Estimating regional contributions (alveolar or airway regions) to decreased FE_{NO} was outside the scope of this study. Further studies assessing FE_{NO} at several flows (e.g., those based on a two-compartment lung model)[22–25] could help establish the relative contribution of alveolar and airway compartments to the fall in FE_{NO} after P_{Emax} maneuvers.

In conclusion, high expiratory pressure decreases FE_{NO} measurements in children. Although repeated spirometry leaves FE_{NO} values unchanged, it could increase variability. Spirometry and P_{Emax} maneuvers should therefore be avoided before measuring FE_{NO}.

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