

# Exhaled Nitric Oxide Following Repeated Spirometry or Repeated Plethysmography in Healthy Individuals

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Subjects with asthma have higher concentrations of exhaled nitric oxide (NO) than normal individuals. It has been demonstrated that in asthmatics, repeated FVC maneuvers reduce NO. Although the cause of this phenomenon is not known, it has been hypothesized that deep breaths associated with FVC maneuvers reduce exhaled NO by affecting neural sources of NO, possibly via a mechanism related to the pathobiology of asthma. To establish whether FVC maneuvers influence NO concentrations in normal individuals, we measured exhaled NO at baseline values and after FVC maneuvers performed every 15 min for 1 h in subjects without asthma. To investigate the role of deep breaths in reducing exhaled NO, we compared these results with concentrations of exhaled NO after plethysmography. Repeated FVC maneuvers over 60 min produced a decrease in NO concentrations in mixed expired gas ( $F_{E}NO$ ;  $24.6 \pm 5.1\%$  decrease for  $F_{E}NO$ ,  $p < 0.01$  versus baseline). In contrast to the results after spirometry, repeated specific airway conductance (sGaw) maneuvers do not have a significant effect on  $F_{E}NO$  ( $p = 0.16$ ). These results, which demonstrate that in nonasthmatic subjects FVC maneuvers—but not panting maneuvers—produce a fall in NO, suggest that the mechanism responsible for the reduction in exhaled NO after FVC maneuvers is related to volume history of the lung rather than the pathobiology of asthma.

In subjects with asthma, it has been demonstrated that repeated forced vital capacity (FVC) maneuvers, such as those that occur during the serial performance of spirometry, produce a 36% reduction in nitric oxide concentrations in mixed expired gas ( $F_{E}NO$ ) (1). This finding has recently been confirmed by Silkoff and colleagues (2). Although the cause of this phenomenon is not known, it has been postulated that the rapid and substantial changes in lung volume that occur during FVC maneuvers lead to a reduction in neurally derived nitric oxide (NO) originating from the lower airway (1). Alternatively, it is possible that the production of NO is influenced by changes in airway caliber known to be induced by deep breaths in asthmatic patients (3, 4). Thus, we would expect that as deep breaths have little influence on airway caliber in normal individuals (3), repeated FVC maneuvers would have no effect on exhaled NO in nonasthmatics. In addition, if such an effect were present in normal individuals, exhaled NO would not fall when pressure and tension fluctuations are prevented by the use of plethysmography rather than repeated FVC maneuvers to measure lung function. We therefore measured exhaled NO in normal individuals after the performance of serial spirometric maneuvers and also examined whether the repeated performance of specific airway conductance (sGaw) maneuvers influenced  $F_{E}NO$  in these patients.

## METHODS

### Subjects

Ten nonsmoking adults without a history of asthma were recruited by advertisement. All were healthy with no history of respiratory illness, had normal spirometry and a history of provocative concentration of methacholine producing a 20% reduction in  $FEV_1$  ( $PC_{20}$ )  $> 8$  mg/ml, and were nonatopic as ascertained by skin prick testing with 10 common aeroallergens. Subjects were not using any medications; caffeine was withheld for 12 h before any testing. The study protocol was approved by the Human Research Committee at the Brigham and Women's Hospital, Boston, Massachusetts. All subjects gave their written informed consent.

### Spirometry Protocol

Subjects underwent exhaled gas collection and spirometry at baseline and every 15 min for 1 h. All study visits began between 8:00 and 10:00 A.M. Spirometry was performed according to American Thoracic Society criteria with a Microlab 3300 spirometer (Micro Medical, Auburn, ME) with subjects in the standing position. At each time point, two FVC maneuvers were performed, and the better value of each set was recorded.

*Exhaled gas collection and NO measurement.* Exhaled gas was collected for NO measurement before spirometry at each time point. During collection, subjects wore nose clips and breathed through a two-way valve apparatus (Hans-Rudolph, Kansas City, MO) that had a pressure transducer attached to a sampling port at the mouthpiece and an instrumental dead space of 120 ml. The output of the pressure transducer appeared on a visible liquid crystal display (LCD) screen located on the analyzer that served as a feedback method allowing the subject to regulate expiratory force (and thus expiratory flow) during exhalation. The intake limb of the valve apparatus was attached to a source of low-NO air (i.e.,  $< 5$  parts per billion [ppb] NO). After 3 to 5 tidal breaths, the subject inhaled to TLC. During this inspiration a Mylar balloon with a restrictor in its neck was attached to the expiratory limb of the valve apparatus. Without a breath hold, the subject exhaled to residual volume (RV) maintaining a mouth pressure of 7 mm Hg, which has been shown to reduce contamination of the exhaled gas with nasally derived air (5); at this mouth pressure, the flow through the valve apparatus and Mylar bag was 385 ml/s. The Mylar bag was subsequently sealed and removed from the expiratory limb of the apparatus. NO measurements were made from the mixed expired gas concentration within the Mylar bag ( $F_{E}NO$ ). The average of three collections at each time was reported.

### Plethysmographic Studies

To assess the effect of plethysmography on  $F_{E}NO$ , all subjects from the spirometry protocol were invited to return to the laboratory for serial measurements of sGaw and  $F_{E}NO$ . Five subjects were able to be recruited and underwent exhaled gas collection and plethysmography at baseline values and every 15 min for 1 h. All study visits began between 8:00 and 10:00 A.M.

*Plethysmography.* To determine sGaw, subjects panted (panting frequency of 1/s) at FRC  $\pm 150$  ml against an open and subsequently shut shutter in a body plethysmograph (BP/PLUS; Warren E. Collins, Braintree, MA). At each time point, two sets of three maneuvers each were performed.

*Exhaled gas collection.* Exhaled gas was collected under similar conditions as during the spirometric studies using a T-piece with one-

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TABLE 1  
CHARACTERISTICS OF THE SUBJECTS IN A STUDY OF EXHALED NO AFTER  
SPIROMETRY AND PLETHYSMOGRAPHY IN NONASTHMATICS

| Subject   | Age (yr)   | Sex | FEV <sub>1</sub> (L)* | Baseline F <sub>E</sub> NO (ppb) | Baseline sGaw† | Baseline Vtg (L)‡ |
|-----------|------------|-----|-----------------------|----------------------------------|----------------|-------------------|
| 1         | 27         | F   | 3.72 (104)            | 8.7                              | 0.28           | 3.59              |
| 2         | 25         | M   | 3.93 (98)             | 9.6                              | 0.17           | 3.88              |
| 3         | 24         | F   | 2.79 (105)            | 5.9                              | 0.36           | 2.50              |
| 4         | 37         | F   | 2.03 (80)             | 8.2                              | —              | —                 |
| 5         | 25         | F   | 3.93 (111)            | 8.4                              | —              | —                 |
| 6         | 27         | M   | 4.54 (113)            | 10.7                             | —              | —                 |
| 7         | 35         | F   | 3.44 (109)            | 6.1                              | —              | —                 |
| 8         | 24         | F   | 3.28 (100)            | 6.4                              | —              | —                 |
| 9         | 20         | M   | 4.72 (108)            | 12                               | 0.18           | 3.80              |
| 10        | 39         | M   | 4.38 (106)            | 6.2                              | 0.26           | 3.66              |
| Mean ± SD | 27.8 ± 2.1 |     | 103 ± 3               | 8.2 ± 0.7                        | 0.25 ± 0.08    | 3.49 ± 0.55       |

\* Values in parentheses represent percent predicted.

† Baseline sGaw in the five subjects who underwent serial measurement of F<sub>E</sub>NO after repeated panting maneuvers.

‡ Baseline volume of thoracic gas (Vtg) in the five subjects who underwent serial measurement of F<sub>E</sub>NO after repeated panting maneuvers.

way valves on the inspiratory and expiratory limbs. The inspiratory limb was attached to a source of NO-free air; the expiratory limb included adapters for a pressure manometer (Mercury Medical, Clearwater, FL) and the Mylar bag connector. This apparatus had an instrumental dead space of 45 ml.

#### Statistics

Data for expired NO during repeated respiratory maneuvers are reported as percentage of baseline value with the standard error of the mean. Changes were identified by one-way and two-way analysis of variance (ANOVA) for repeated measures, with Dunnett's *post hoc* procedure where appropriate (Sigmastat; Jandel Scientific, San Rafael, CA).

#### RESULTS

##### Main Protocol

**Subjects.** Eleven subjects were recruited. One was not able to comply with study procedures and was excluded from analysis. The demographic, baseline NO values, and baseline spirometry data for the 10 study subjects are presented in Table 1.

**Expired NO and FEV<sub>1</sub>.** Baseline NO values in mixed expired gas are shown in Table 1. In nonasthmatics, repeated FVC maneuvers produced a drop in F<sub>E</sub>NO (ANOVA  $p < 0.01$ , Figure 1). Compared with the pre-FVC baseline value, a decline in F<sub>E</sub>NO occurred within 15 min after the first set of FVC maneuvers ( $-16.3 \pm 6.4\%$ ,  $p < 0.01$ ). The maximal decrease in F<sub>E</sub>NO occurred at 60 min, after the subjects had performed eight sets of FVC maneuvers over a 60-min sampling interval. F<sub>E</sub>NO fell  $23.4 \pm 5.0\%$  compared with baseline,  $p < 0.01$ . In contrast, repeated forced expiratory maneuvers produced no change in FEV<sub>1</sub> over the observation period in these subjects (one-way ANOVA,  $p = 0.29$ , Figure 1).

##### Plethysmographic Studies

**Expired NO.** In the five subjects who returned to the laboratory for plethysmography, F<sub>E</sub>NO values after panting maneuvers were compared with F<sub>E</sub>NO values previously obtained from these same subjects during serial spirometry. In contrast to the effect noted with spirometry, serial sets of panting maneuvers at FRC did not produce a significant decrease in exhaled NO (Figure 2, F<sub>E</sub>NO postplethysmography). Compared with baseline, F<sub>E</sub>NO values ranged from  $+20.9 \pm 19.6\%$  at 30 min to  $-3.5 \pm 2.5\%$  60 min after panting maneuvers began (ANOVA,  $p = 0.16$ ,  $\beta = 0.78$ ). Similarly sGaw values ranged from  $+16 \pm 10.6\%$  at 30 min to  $+4.6 \pm 8.7\%$  15 min after panting maneuvers began (ANOVA,  $p = 0.25$ ,  $\beta = 0.87$ , Figure 2). These data were compared with the exhaled NO data

previously obtained for these five subjects after serial spirometry. F<sub>E</sub>NO values after serial spirometry were lower than F<sub>E</sub>NO levels measured after serial sGaw determinations (two-way ANOVA,  $p = 0.02$ ).

#### DISCUSSION

Despite the fact that NO is easy to detect in exhaled gas, multiple factors affect the concentrations measured. Disease states such as asthma, bronchiectasis, and respiratory infection are associated with increased concentrations of exhaled NO (6–9). Pulmonary hypertension, corticosteroid use, and tobacco smoking are associated with decreased levels of exhaled NO (6, 10, 11). In addition, collection techniques have a significant impact on measured NO values (8–10, 12–16); it is therefore important to control for these factors which include expiratory flow rate, lung volume, expiratory pressure, and time of day (17, 18). It has been demonstrated that in subjects with asthma repeated FVC maneuvers reduce F<sub>E</sub>NO (1, 2). We set

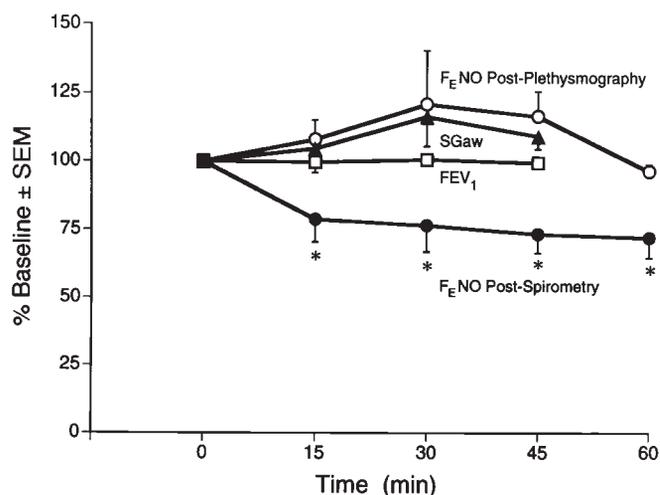
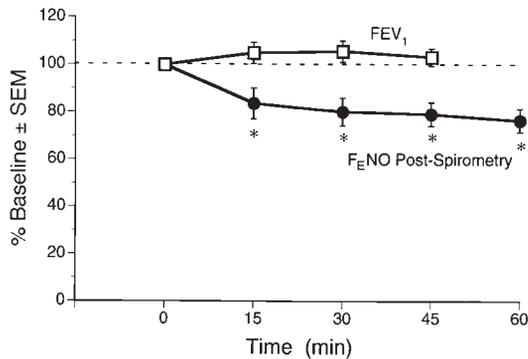


Figure 1. Effect of repeated spirometry on F<sub>E</sub>NO and FEV<sub>1</sub> in 10 nonasthmatic subjects. NO was measured before FVC maneuvers at baseline values and every 15 min for 1 h. Repeated FVC maneuvers produced a significant reduction in F<sub>E</sub>NO (solid circles, one-way ANOVA  $p < 0.01$ ). In contrast, repeated FVC maneuvers had no effect on FEV<sub>1</sub> (open squares). \* $p < 0.01$  for specific time points as compared with baseline for F<sub>E</sub>NO.



**Figure 2.** Effect of repeated panting maneuvers on  $F_{E}NO$  and  $sGaw$  in five nonasthmatic subjects in comparison to the effect of repeated spirometry on  $F_{E}NO$  and  $FEV_1$  previously obtained from these five individuals. NO was measured before FVC or panting maneuvers at baseline values and every 15 min for 1 h. Repeated panting maneuvers produced no significant change in  $F_{E}NO$  (open circles, one-way ANOVA  $p = 0.16$ ) or  $sGaw$  (solid triangles, one-way ANOVA  $p = 0.25$ ). In contrast, previous data from these five individuals demonstrated that repeated FVC maneuvers produced a significant reduction in  $F_{E}NO$  (solid circles, one-way ANOVA  $p < 0.01$ ) while producing no change in  $FEV_1$  (open squares, one-way ANOVA  $p = 0.47$ ). \* $p < 0.01$  for specific time points as compared with baseline for  $F_{E}NO$  postspirometry.

out to establish whether this phenomenon is observed in subjects without asthma and to determine which respiratory maneuvers affect exhaled NO. Our data demonstrate that in healthy, nonasthmatic subjects repeated FVC maneuvers do indeed reduce expired NO, and the majority of this reduction occurs after the first set of two FVC efforts. Furthermore, we have found that repeated measurement of airway conductance by plethysmography does not reduce mixed expired NO.

Our present data extend the earlier findings that serial FVC maneuvers reduce  $F_{E}NO$  in asthmatic patients (1). Repeated FVC maneuvers reduce  $F_{E}NO$  by approximately 20% in normal individuals, which is similar to the effect in asthmatics and in agreement with Silkoff and colleagues who recently reported a 13% decrease in exhaled NO 1 min after three FVC maneuvers (2). Our finding that this reduction occurs in nonasthmatics suggests that the mechanism of the reduction is not linked to the pathobiology of asthma, but appears to be a more general phenomenon related to the volume history of the lung. It is interesting to note that the majority of the reduction in NO occurs after the first set of FVC maneuvers, which suggests that the source of this reduction is completely suppressed by these maneuvers. Whether the magnitude of this effect changes from day to day, and whether the magnitude of such a change is similar in asthmatics and normal individuals is not known and warrants further investigation.

Support for modulation of exhaled NO by intrathoracic pressure and volume changes has been reported by Persson and colleagues, who found increased exhaled NO in anesthetized rabbits after application of positive end-expiratory pressure (PEEP) (19). The finding that the increase in exhaled NO induced by PEEP was diminished by vagotomy indicates the central role of neural pathways in mediating this effect. Further, data specifically supporting a reduction in exhaled NO with respiratory maneuvers have been reported by Stromberg and colleagues, who found that application of positive extrathoracic pressure, a maneuver likely to produce volume and pressure changes similar to an FVC maneuver, results in a decline in exhaled NO in a rabbit model (20).

Although the limited size of our study does not allow us to definitively conclude that plethysmography has no effect on

$F_{E}NO$ , there was no significant difference detected, and the trend for any change induced by plethysmography was in the opposite direction as that caused by FVC maneuvers. If plethysmography does indeed cause a slight increase in  $F_{E}NO$ , this effect may be mediated by the modest trend we found for increases in airway conductance with repeated assessment. This increase closely tracked the change in  $F_{E}NO$ , and such a relationship between airway caliber and  $F_{E}NO$  is consistent with the findings of Silkoff and colleagues who have reported that administration of a bronchodilating agent produced approximately a 10% increase in exhaled NO (2). In contrast, our data, which demonstrate no change in  $FEV_1$  during serial assessment, indicate that the decrease in  $F_{E}NO$  seen with repeated FVC maneuvers is not the result of reductions in airway caliber induced by spirometry.

In summary, we have documented a reduction in exhaled NO produced by FVC maneuvers in subjects without asthma. Repeated plethysmography does not produce this effect, thereby suggesting it is related to the pressure and volume history of the lung. As plethysmography does not significantly perturb  $F_{E}NO$ , these measurements may be the preferred method of assessing respiratory function while serially gathering information about exhaled NO.

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