

The effect of spirometry and exercise on exhaled nitric oxide in asthmatic children*

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American Thoracic Society (ATS) guidelines recommend to refrain from spirometry or exercise before measuring fractional exhaled nitric oxide (FENO) because forced breathing maneuvers might influence FENO values. However the few studies already reported in children have given conflicting results. The aim of the study was to observe to what extent spirometry or exercise could affect FENO in asthmatic children. Twenty-four asthmatic children (mean age 12.8 yr) were enrolled. Measurements of FENO were performed before and 5, 15, 30, 45 and 60 min after spirometry or a 6-min walk test, on two separate days in random order. Geometric mean FENO at baseline was 25.6 parts per billion (ppb) before spirometry and 23.5 ppb before exercise. A small drop of FENO to 24.2 and 23.7 ppb was found 5 and 15 min after spirometry (both $p = 0.04$). After exercise, FENO values showed a larger drop to 18.5 ppb after 5 min and 20.7 ppb after 15 min ($p < 0.001$; $p = 0.004$ respectively). Changes in FENO occurred after exercise irrespective of baseline FENO and values returned to baseline within 30 min. We conclude that both spirometry and exercise affect FENO in asthmatic children. As the changes after exercise may lead to erroneous interpretations, children should refrain from physical exercise during at least 30 min before FENO measurements.

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Fractional exhaled nitric oxide (FENO) is an easy, repeatable, safe and non-invasive marker of bronchial inflammation in allergic asthma. Standardized guidelines for the measurement of FENO have been published, and analyzers that meet those requirements are commercially available for clinical application (1, 2). Guidelines from the joint European Respiratory Society (ERS)/American Thoracic Society (ATS) task force recommend to perform FENO analysis before spirometric tests, because it has been shown that forced expiratory maneuvers reduce FENO levels in healthy and asthmatic adults (3–5). However, only one study by Kissoon et al. (6) is available on the effect of spirometric tests on FENO in children and the results are in contrast to those found in adults by Silkoff et al. (4) and Deykin et al. (3, 5). Exercise may reduce FENO values as well (7–11) and guidelines recommend to refrain from strenuous exercise

for 1 h before the FENO test. Again, the few data that are available on children reached conflicting results (12,13). In particular, while a study by Scollo et al. (12) reported no change in FENO values after a treadmill exercise challenge, Terada et al. (13) reported that FENO values significantly decreased after exercise in children with and without exercise-induced bronchoconstriction (EIB) and in healthy subjects. FENO is increasingly used next to spirometry, hence it seems of practical importance to understand whether forced expiratory maneuvers influence FENO values in children. Also, children are likely to run and play in the waiting room, thus it is important to understand to what extent physical exercise can influence their FENO values. The duration of the effects of both spirometry and exercise on FENO values is not yet known, therefore we established the timing of the measurements in relation to such maneuvers.

The aim of the present study was to observe whether or not spirometry or exercise immediately preceding FENO measurement could influence FENO values in asthmatic children.

Materials and methods

Subjects

We recruited 24 asthmatic children (12 boys) aged 7.9–17.7 yr (mean 12.8 yr) with a range of FENO values [6.5–176.2 parts per billion (ppb)]. A diagnosis of asthma was based on a history of recurrent episodes of wheezing, coughing and shortness of breath, reversible bronchoconstriction and/or airway hyperresponsiveness to methacholine (14).

Seventeen subjects had RAST class 2 or higher for at least one airborne allergen and all patients were on controller medication at the moment of the study (Table 1). All subjects used inhaled salbutamol as needed. Bronchodilators were withheld for 8 h (short acting) or 48 h (long acting) before the tests.

None of the patients had a history of upper respiratory tract infection or asthma exacerbation during 2 wk before the study. Parents and children, if older than 12 yr, gave their written informed consent. The study was approved by the Medical Ethical Committee of the Erasmus MC–Sophia Children's Hospital.

Study design

The study was performed on 2 days within 2 wk. In random order, children performed spirometry (day A) or a 6-min walk test (day B). On both days baseline FENO was measured twice during a resting period of 30 min, at $t = -30$ min and $t = -15$ min. Then either exercise or spirometry was performed ($t = 0$), followed by FENO measurements at $t = 5, 15, 30, 45$ and 60 min.

Pulmonary function testing

Spirometry was performed with a dry rolling seal spirometer (Jaeger, Würzburg, Germany), according to ATS criteria (15). Three forced vital capacity (FVC) maneuvers were performed and the best value of FVC and forced expiratory volume in 1 s (FEV₁) was recorded.

Exercise testing

A 6-min walk test was performed indoors along a long, flat, straight, enclosed corridor with a hard surface (16). The test was modified and adapted to the study needs; children walked between two 8-m points for 6 min. Heart rate was recorded at the start and at the end of the exercise; the total number of rounds and the total meters covered were recorded.

Table 1. Baseline characteristics of study subjects

Subject	Age (yr)	Sex	FENO baseline pre-spirometry*	FENO baseline pre-exercise*	FEV ₁ % of predicted	FVC % of predicted	Allergy (RAST class 2 or higher)	Controller medication mcg/day
1	9	M	11.2	15.4	112	126	No	Budesonide 400, Formoterol 12
2	11	M	18.0	12.8	81	83	Dust mite	Beclometasone 400
3	11	F	36.2	25.5	116	108	No	Budesonide 400
4	15	M	14.8	9.9	85	104	Dust mite pollens	Budesonide 400
5	11	M	8.5	6.9	100	100	Dust mite pollens	Budesonide 400, Formoterol 12
6	17	F	46.7	29.4	109	116	Pollens	Budesonide 400, Formoterol 9
7	11	F	7.2	8.3	113	111	Dust mite	Fluticasone 200
8	12	M	45.8	95.8	94	93	Dust mite	Budesonide 100
9	9	F	14.1	13.0	105	117	No	Budesonide 400, Formoterol 12
10	12	M	33.0	45.9	77	96	Grass Pollens	Fluticasone 200, Salmeterol 50
11	14	M	28.6	19.3	93	80	Dust mite	Budesonide 400
12	14	F	58.8	105.3	92	107	Dust mite	Budesonide 400
13	13	M	27.2	10.1	99	108	Dust mite pollens	Budesonide 400, Formoterol 12
14	12	F	176.2	62.9	105	104	No	Fluticasone 200
15	15	M	140.3	85.3	103	98	Dust mite pollens	Budesonide 400, Formoterol 12
16	11	F	6.5	8.2	92	78	No	Fluticasone 200, Salmeterol 50
17	10	M	14.8	13.5	99	108	Dust mite	Beclometasone 400
18	14	F	78.4	77.4	77	68	Dust mite pollens	Fluticasone 250
19	14	F	35.0	19.4	105	100	Dust mite pollens	Beclometasone 400, Formoterol 12
20	16	F	32.2	39.0	109	97	No	Beclometasone 400
21	13	F	17.9	14.7	107	101	Dust mite cat dog	Beclometasone 400
22	7	M	17.6	35.1	75	77	Dust mite pollens	Beclometasone 400
23	16	F	27.5	32.0	103	107	No	Budesonide 400, Formoterol 9
24	10	M	11.1	14.2	76	112	Dust mite	Beclometasone 400

*FENO is expressed as geometric mean of two triplicate measurements.

FENO measurements

The FENO measurements were carried out with the NIOX NO-analyzer (Aerocrine, Stockholm, Sweden) according to ERS/ATS guidelines (2). Children inspired NO-free air and exhaled for a minimum of 7 s. Exhalation flow was kept constant at 50 ml/s through a visual feedback mechanism and dynamic flow restrictor. Any exhalation, which did not meet the ERS/ATS requirements, was not accepted by the NIOX system and the subjects were asked to perform a new exhalation maneuver. At each session three correctly executed exhalations were recorded. FENO values were expressed in ppb.

Statistical analysis

Intraclass correlation coefficients were calculated for both days in order to assess the reproducibility of FENO at $t = -30$ and $t = -15$. Differences in baseline values of the two study days were analyzed by paired t -test. Changes in FENO from baseline at the various time points were analyzed with repeated measurements ANOVA; two-tailed p -values of <0.05 were considered significant.

The FENO data were log-transformed before analysis in order to obtain a normal distribution, and expressed as geometric mean. Regression analysis was used to investigate the relation between FEV₁ and FENO.

Results

Baseline FENO before spirometry and exercise were not significantly different and the two baseline measurement were highly reproducible within children (intraclass correlation coefficient = 0.99 on both days). Hence, the geometric mean of the two baseline values was calculated and used as the individual baseline for the analysis. Baseline geometric mean FENO values were 25.6 ppb (range 6.5–176.2 ppb) before spirometry and 23.5 ppb (range 7.0–105.3 ppb) before exercise.

All subjects successfully performed the spirometric tests. Mean FEV₁ was 97% of predicted (range 75–116%), and mean FVC was 100% of predicted (range 68–126%). A small but significant drop of FENO to 24.2 ppb and to 23.7 ppb was found, respectively, 5 and 15 min after spirometry (both $p = 0.04$; Fig. 1). Values of FEV₁ did not significantly correlate either with the baseline FENO or with changes in FENO.

Children covered an average distance of 473 m (range 272–848 m), leading to a mean increase in

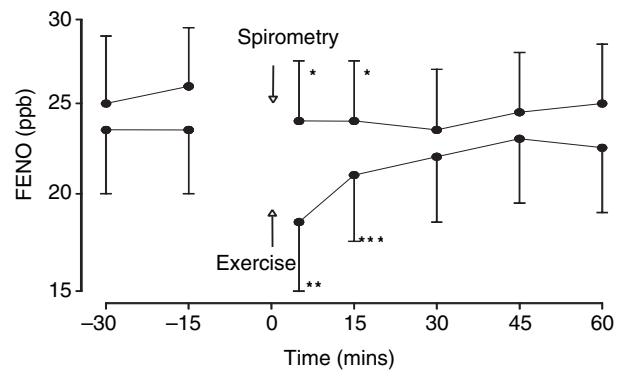


Fig. 1. FENO before and after spirometry (upper curve) and exercise (lower curve) in asthmatic children ($n = 24$). FENO is shown as geometric means and SEM. The changes in log-transformed FENO values 5 and 15 min after spirometry and exercise, compared to baseline values, are significant (* $p = 0.04$, ** $p < 0.001$, *** $p = 0.004$).

heart rate of 87 beats per minute (range 31–133). We found a consistent and significant drop in FENO values after exercise to 18.5 ppb after 5 min and 20.7 ppb after 15 min ($p < 0.001$ and $p = 0.004$ compared with baseline respectively) (Fig. 1). Nineteen subjects (79%) showed the maximum drop in FENO values within 5 min after exercise. A fall in FENO after exercise occurred irrespective of baseline FENO and independently of the amount of exercise as reflected by the increase in heart rate (Fig. 2). Only two subjects did not show a recovery of FENO values within 80% of baseline within 30 min after exercise. The mean changes in FENO from baseline at 5 min after spirometry and exercise were significantly larger after exercise than after spirometry ($p < 0.001$, paired t -test).

Discussion

This is the first study comparing the effects of spirometry and exercise on FENO in asthmatic children. Our results show a consistent, significant reduction of FENO both after exercise and after spirometry. The effect of exercise on FENO values is larger than that of spirometry and the changes of FENO values are seen in the first 30 min after these maneuvers.

Previous studies about the influence of either spirometry or exercise on FENO values reached different conclusion. Deykin et al. (3) studied asthmatic adults and found that spirometry induced a significant drop in FENO values that was maximal 30 min after spirometry. The authors speculated that repeated spirometric maneuvers reduced NO production through an

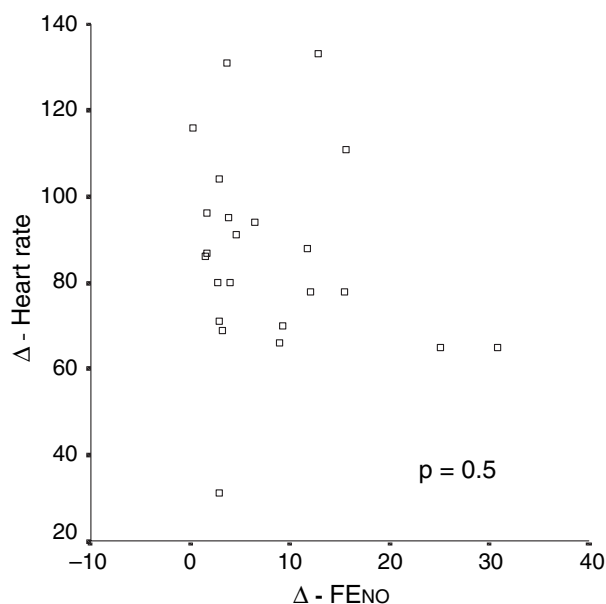


Fig. 2. Changes in FENO values (Δ - FENO) after exercise in relation to the amount of exercise, expressed as difference in heart rate post and pre - exercise (Δ - heart rate). There is no significant correlation.

effect on bronchodilating non-adrenergic non-cholinergic nerves within the airways (3). Silk-off et al. (4) found a fall in FENO values 1 min after spirometry in normal and asthmatic adults. In both groups, FENO returned to baseline within 1 h. In another study, Deykin et al. (5) documented a reduction in FENO after FVC maneuvers in non-asthmatic adults, while no change was found after repeated plethysmography, and suggested that the change in FENO values after spirometry is related to the pressure and volume history of the lung. Surprisingly, the only study on the effect of spirometry on FENO in children reached the opposite conclusion. Kissoon et al. (6) observed that FENO values slightly increased within 8–18 min after spirometry in asthmatic children. In this study five of 10 asthmatic children used inhaled steroids, and in children on steroids, the mean increase of FENO was lower than in children not on steroids. Authors also evaluated the effect of spirometry and metered dose inhaler (MDI)-placebo on FENO and found that in poorly controlled asthmatics (not on inhaled steroids) there was a significant increase of FENO values 18 min after the study maneuvers. They speculated that the rapid and substantial changes in lung volume that occur during spirometry may trigger neural mechanisms leading to increased NO release from the lower airway (6).

Our results are in agreement with the findings previously reported in adults, while different from the results of the study of Kissoon et al. The reasons for the discrepancies are not clear. The use of inhaled corticosteroids by the participants of our study might have suppressed the NO production from the source of nitric oxide, which may be sensitive to forced breathing. This could explain, on the one hand, the opposite results from the study of Kissoon et al., and also the relatively small decrease of FENO levels after the forced expiratory maneuvers, which seems unlikely to be of clinical relevance in most situations, particularly as the fall in FENO was observed to the greatest extent in children with normal levels.

Our findings regarding the effect of exercise on FENO are consistent with earlier observations. Data reported by Terada et al. (13) showed a drop in FENO values after exercise in asthmatic and healthy children. These authors also observed that patients with EIB were more likely to need a longer recovery than children without EIB before FENO values came back to baseline (13). In contrast, Scollo et al. (12) evaluated the effects of exercise on FENO levels in 24 asthmatic children and 18 healthy controls; they concluded that FENO values did not change after exercise challenge in either healthy controls or in asthmatic children with and without EIB (12). Exercise may lead to hyperventilation and reduced airway caliber in asthmatic children; both increase airflow velocity. We speculate that changes in bronchial diameter are responsible for reduced FENO levels after exercise. To avoid repeated forced breathing, we did not measure lung function after exercise. Despite a constant exhalation flow during FENO measurements, the reduced airway surface area might have led to lower nitric oxide diffusion through the airways. We found a recovery to baseline FENO values after 30 min post-exercise, when there was no clinical evidence of hyperventilation or bronchoconstriction.

What are the clinical implications of our findings? Children play and run in the waiting room. They should however refrain from exercise before performing FENO measurements, or FENO values would be underestimated, depending on the amount of exercise performed. We also defined the duration of the effect of exercise on FENO values and we suggest that asthmatic children refrain from strenuous exercise for 30 min before the FENO measurements, rather than the recommended 60 min. In fact, beyond 15 min there is only a small variation in FENO values, which can be attributed to random variation with time.

Although the effect of spirometry on FENO appears small, we suggest that future guidelines recommend to refrain from spirometry for 30 min before FENO measurements.

We conclude that exercise and, to a lesser extent, repeated spirometric maneuvers influence FENO values in children. As the changes after exercise may lead to erroneous interpretations, children should refrain from physical exercise during at least 30 min before FENO measurements.

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