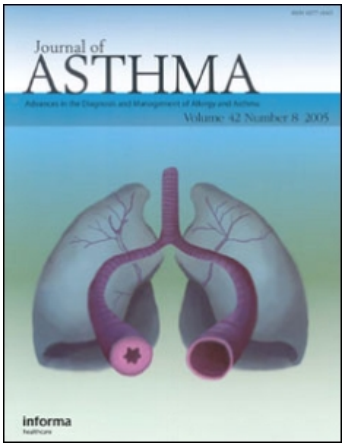

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ORIGINAL ARTICLE

Variations in Exhaled Nitric Oxide in Children with Asthma During a 1-Week Stay in a Mountain Village Sanatorium

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Knowing about spontaneous variations in the fractional concentration of exhaled nitric oxide (FE_{NO}) could improve monitoring of airway inflammation in asthmatic children. We aimed to assess FE_{NO} variations (expiratory flow 50 mL/sec) in subjects maintained in similar environmental conditions. We tested spirometry and FE_{NO} in symptom-free asthmatic children (9 corticosteroid-naive, 8 corticosteroid-treated) during a 1-week stay in a countryside sanatorium and in their healthy relatives (n = 12) staying in the immediate neighborhood on summer holiday (total 29 children, M/F:14/15, 5.8-16.8 yrs). Testing sessions were repeated every 12 hours (8:00 AM, 8:00 PM) for 2 days and again on day 7. Measurements were defined as reproducible when they agreed with an intraclass correlation coefficient (ICC) above 0.60; deviation from mean differences was assessed by the coefficient of repeatability (CR = 2 SD). Lung function remained constant throughout the week in all groups. Baseline FE_{NO} levels in corticosteroid-naive asthmatic children tended to decrease at the end of the week (from 13.9 ppb, 95% CI 12.2-19.1 to 9.2 ppb, 95% CI 5.8–15.9, $p = 0.057$). No differences were found between nocturnal and diurnal FE_{NO}. Within-session reproducibility for two FE_{NO} measurements was high (ICC 0.99 in all groups and CR, 0.9 to 1.3 ppb). Between-session FE_{NO} reproducibility at 12 hours and 24 hours was still high for each group but decreased markedly after 6 days in corticosteroid-naive asthmatic children (ICC 0.79 and CR 9.6 ppb at 24 hours *vs.* ICC 0.13 and CR 20.8 ppb after 6 days), whereas it decreased slightly in corticosteroid-treated asthmatics (from ICC 0.89 and CR 3.1 ppb to ICC 0.88 and CR 3.0 ppb) and healthy children (from ICC 0.79 and CR 4.8 ppb to ICC 0.65 and CR 5.7 ppb). In conclusion, in healthy subjects and in asthmatic children receiving therapy with inhaled corticosteroids (but not in corticosteroid-naïve subjects), FE_{NO} measurements are reproducible across a week.

Keywords exhaled nitric oxide, asthma, repeatability, children

INTRODUCTION

Airway inflammation, especially in asthmatic children, is now widely monitored by measuring the fractional concentration of exhaled nitric oxide (FE_{NO}). Several studies in patients with asthma report that FE_{NO} variations probably reflect either loss of disease-control or response to corticosteroid therapy (1–9). In addition, several cut-off values of FE_{NO} have been proposed as predictors of asthma relapse after corticosteroid withdrawal or exacerbations during therapy (1, 3, 10–12). How large an increase in FE_{NO} (using similar on-line measures) is necessary to discriminate changes due to asthma relapse (or exacerbations) from the random variation in this marker remains unclear. A first step towards answering this question is to calculate the spontaneous variations in FE_{NO} measurements over time.

Previous studies investigating spontaneous variations in FE_{NO} measurements in corticosteroid-naïve or corticosteroid-treated children were mostly conducted in outpatients (13–17). FE_{NO} measurements in outpatients could nevertheless be influenced by between-individual differences in the ambient where patients live. FE_{NO} measurements in healthy children could also be influenced by environmental differences, although previous studies found them reproducible (17–20). No study has yet confirmed reproducible FE_{NO} measurements at the currently recommended expiratory flow of 50 mL/sec (21) in children maintained in similar environmental conditions, for example in a health care center or a countryside sanatorium. Knowing more about spontaneous FE_{NO} variations over time is important to help improve monitoring of patients with asthma.

We compared spontaneous FE_{NO}variations over time in asthmatic children (with vs. without inhaled corticosteroid therapy) and healthy children living in comparable ambient conditions. To this purpose, we tested lung function (spirometry) and FE_{NO} in symptom-free asthmatic children during a 1-week stay in a countryside sanatorium and in their healthy relatives staying in the immediate neighborhood on summer holiday. In these groups we also investigated whether random variations in FE_{NO} levels would remain within a target of reproducibility as defined by the intraclass correlation coefficient (ICC).

METHODS

Subjects

From consecutive children with asthma hospitalized at the Srobar Institute for Respiratory Diseases (Dolny Smokovec,

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Abbreviations: FE_{NO}, fractional concentration of exhaled nitric oxide; CV, coefficient of variability; CR, coefficient of repeatability; ICC, intraclass correlation coefficient

Tatry, Slovak Republic) and their healthy relatives staying nearby we invited 32 children (12 healthy subjects and 20 children with asthma) to participate in a 1-week study. The Srobar sanatorium is situated in a wooded area near the Tatra mountains at an altitude of 970 m. The ambient temperature in the summer ranges from 18° to 20°C at night, and from 22° to 24°C in the morning, humidity ranges from 70% to 80%. In this Institute, children with chronic respiratory conditions follow brief diagnostic-therapeutic programs, consisting of medication, physical exercise (walking, gymnastics, games), physiokinesotherapy, and training for asthma self-management. Healthy children were patient's relatives staying close to the sanatorium during the same school-holiday. Asthmatic and healthy children were diagnosed as described elsewhere (22). The Asthma Control Test (ACT) was used to select subjects with good disease control; ACT scores were monitored during the study (23). Inclusion criteria were good asthma control in the past 4 weeks (negative for limitation of activities, daytime symptoms, nocturnal symptoms/awakening, and need for rescue medication) and absence of respiratory infections in the preceding 4 weeks. Of the 20 asthmatic children who participated, 13 had a history of atopy and were sensitized to multiple inhaled allergens (mainly house dust mites but also cat or dog fur, grass or tree pollens, and molds). Ten asthmatic children received continuous inhaled corticosteroid therapy (fluticasone 125 μg or budesonide 200 μg twice a day) for at least 1 month. Informed consent was obtained from parents and the study was approved by the Hospital Ethics Committee.

Study Design

On arrival at the sanatorium all patients underwent a clinical examination, and a history was recorded of symptoms and therapy. At 8:00 PM and every 12 hours for 2 days and again on day 7 at 8:00 AM subjects underwent lung function testing and FE_{NO} measurements. Participants were excluded from the study if their cooperation with the maneuvers was considered unsatisfactory (unable to provide acceptable and reproducible duplicate lung function or FE_{NO} maneuvers as recommended) (21, 24) or respiratory symptoms developed during the weekly follow-up. Healthy participants living near the sanatorium underwent the same assessments.

Lung Function Measurements

Spirometry. Forced vital capacity (FVC) maneuvers were recorded with a portable spirometer (heated Fleisch pneumotachograph ESS-γ, Biomedin s.r.l., Padova, Italy) with subjects in the standing position. Duplicate measurements from at least three acceptable maneuvers were obtained and recorded as recommended (24). Values were expressed as percent of predicted (25).

FE_{NO} measurements. FE_{NO} was measured by chemiluminescence (Sievers NOA 280; Sievers Instruments Inc., Boulder, CO, USA) as previously reported (26). The single-breath on-line technique with inhalation of NO-free air was used (27). All participants were asked to avoid food intake and physical exercise for at least 2 hours before testing. Subjects did single breath maneuvers in duplicate at a constant expiratory pressure and flow (10 cm H_2O and 50 mL/s) as described in detail elsewhere (26). An effort was accepted when the expiratory pressure fluctuated less than $\pm$2 cm H_2O (from 10 cm H_2O) during the maneuver (14). Subjects maintained a single exhalation until they did a FE_{NO} plateau of at least 4 seconds. Duplicate exhalations were accepted when measurements agreed within 10% (21). In all cases FE_{NO} measurements preceded FVC maneuvers.

Low ambient averaged-NO levels were recorded during sessions (range 1.5 to 9 ppb).

Statistical analysis. FE_{NO} values were non-normally distributed (Kolmogorov-Smirnov test) and 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 Kruskal-Wallis test was used to assess between-group measurement differences for each session. Nonparametric ANOVA (Friedman test) with post hoc Dunnett test was used to assess significant within-group differences between measurements obtained at each step against measurements at baseline. The within-subject coefficient of variation (CV = SD/mean) was used to assess variability in FE_{NO} measurements across five sessions. The Bland–Altman method was used to assess the coefficient of repeatability (CR=2 SD from the mean difference) between two within-session or between-session FE_{NO} measurements (28). Reproducibility was assessed by means of the ICC; this coefficient estimates the average correlation between all possible ordering of pairs of measures (two within-session or between-session FE_{NO} measurements), where an ICC value of 1 denotes perfect reproducibility and a value of 0 denotes no different reproducibility than expected by chance (29). An ICC target of at least 0.6 for repeated measures was accepted as clinically useful (30).

All FE_{NO} measurements (from five sessions) were used for calculations of within-session CR and ICC. The number of FE_{NO} measurements used for calculations of between sessions CR and ICC was drawn by multiplying the number of subjects (n) by the number of session intervals as follows: n $\times$ 2 for 12-hour (two night-to-morning intervals) and 24-hour intervals (first *vs*. second night and first *vs*. second morning), n $\times$ 1 for 6 days interval (first vs seventh morning).

A statistical software (SPSS 10, Chicago, Ill) was used for calculations. p values less than 0.05 were considered statistically significant.

RESULTS

A total 29 children (M/F:14/15, mean age 10.7 $\pm$ 2.6 years, range 5.8–16.8) completed the tests (12 healthy, 9 corticosteroid-naive asthmatics, 8 corticosteroid-treated asthmatics) (Table 1); all 17 asthmatic children remained in good disease control. Two children, 1 corticosteroid-naive and 1 corticosteroid-treated child, were unable to cooperate. A third child (steroid-treated) had fever and chills on the second day of the study and was excluded.

Between-group comparisons at baseline (Kruskal-Wallis test) showed the lowest lung function values and the highest FE_{NO} levels in corticosteroid-treated asthmatics; between-group differences on lung function remained unchanged at each session, whereas differences on FE_{NO} lost statistical significance at the last session (day 7) (Table 2).

TABLE 1.—Demographic and clinical characteristics in the study sample of 29 children.

Characteristics	Healthy (n = 12)	Asthmatics (n = 17)	
		Untreated (n = 9)	Treated (n = 8)
Age (yrs)	10.1 (8.5–11.7)	10.9 (9.0–12.8)	10.3 (8.1–12.4)
Gender (M/F)	5/7	4/5	5/3
Atopy (n)			
–House dust mites	–	3	2
–Cat hair	–	1	0
–Molds	–	1	1
–Grass pollen	–	3	3
–Tree pollen	–	0	1
–Positive for at least one allergen	–	5	5
Duration of asthma (yrs)	–	6.3 (4.2–8.3)	7.6 (4.3–10.9)
Asthma in the past 12 months (n)	–	1	4
Exercise-induced asthma (n)	–	5	5
FEV_1 (%)	101.3 (95.1–107.5)	94.6 (87.8−101.4)	87.5* (78.7–96.4)
FVC (%)	99.2 (91.4–106.9)	91.9 (86.5−97.3)	93.8 (80.2–107.3)
FEF_{25-75} (%)	97.0 (81.6−112.3)	92.9 (76.6–109.4)	80.4 (53.1–107.8)
FE_{NO} (ppb)	6.9 (5.5–8.6)	13.9** (12.2–19.1)	7.1 (5.3–9.5)

*$p < 0.05$ and **$p < 0.005$ vs. healthy subjects by Kruskal-Wallis test.
Spirometric variables are expressed as percent of predicted. Values are geometric (FE_{NO}) or arithmetic means (age, duration of asthma, spirometry) and 95% confidence intervals.

Among-group comparisons (analysis of variance [ANOVA] Friedman with post hoc Dunnett test) showed constant lung function values throughout the week, whereas FE_{NO} levels tended to decrease among corticosteroid-naive asthmatic children, although the decrease reached only borderline statistical significance ($p = 0.057$) (Table 2). FE_{NO} and lung function measurements were found unrelated (data not presented).

No differences were found between nocturnal and diurnal FE_{NO}, although levels appeared higher at the first session (first night) than at the second session (first morning) (8.6 *vs.* 7.5 ppb, mean ratio 1.1, $p = 0.068$).

The mean FE_{NO} coefficient of variation for five sessions was similar in the three groups (27.6% for steroid-naive asthmatics, 21.3% for corticosteroid-treated asthmatics, and 24.5% for healthy children).

Within-session reproducibility and repeatability for two FE_{NO} measurements were high in the 3 groups (ICC = 0.99 in all groups and CR 0.9 to 1.3 ppb) (Figure 1). High reproducibility, i.e., an ICC close to 1.0 meant an almost perfect averaged correlation between two within-session FE_{NO} measures, whereas high repeatability, i.e., low CR values (0.9 to 1.3 ppb depending on the group) meant narrow deviation from mean difference between two within-session FE_{NO} measures. Between-session FE_{NO} repeatability at 12 hours was still high for each group and did not differ from that estimated at 24 hours. After 6 days, FE_{NO} repeatability decreased markedly (low ICC and high CR values), particularly in corticosteroid-naive asthmatic children (Table 3). In these untreated asthmatic children, the percentage CR (expressed as 2 SD from the percent mean difference) for FE_{NO} measurements between the first and the second morning was 69.2% and increased to 128% between the first and the seventh morning. In contrast, percentage FE_{NO} CRs for the same morning intervals increased slightly in both groups (in corticosteroid-treated asthmatics from 44.8% to 48.0% and healthy children from 60.6% to 78.4%) (Figures 2A and 2B).

DISCUSSION

This study show distinct FE_{NO} variations over time in symptom-free asthmatic and healthy children living under comparable ambient conditions (countryside sanatorium and in the immediate neighborhood on summer holiday). At the end of the 1-week study, unlike corticosteroid-treated asthmatics and healthy children, corticosteroid-naïve asthmatic children failed to achieve the target of reproducibility, namely an ICC of more than 0.6. Corticosteroid-naïve asthmatic children can therefore be expected to have poorly reproducible FE_{NO} levels over a week even if their lung function remains unchanged.

The high within-session reproducibility we found in symptom-free asthmatic children fits in with previous reports in healthy and asthmatic children regardless of whether they received inhaled corticosteroid therapy (13–20). Repeated FE_{NO} measurements in a single session agree with ICC values above 0.98 (14, 20, 26).

In this study, to investigate within-day and between-day FE_{NO} reproducibility we measured FE_{NO} 12 and 24 hours

TABLE 2.—Measurements of exhaled nitric oxide (FE_{NO}) and lung function.

Subjects	1st night	1st morning	2nd night	2nd morning	7th morning
Healthy (n = 12)					
FE_{NO} (ppb)	6.9 (5.5−8.6)	5.9 (4.5−7.8)	6.6 (5.1−8.6)	7.0 (5.3−9.2)	7.1 (5.6−9.1)
FEV_1 (%)	101.3 (95.1−107.5)	100.2 (95.4−105.0)	100.0 (94.3−105.7)	96.4 (91.5−101.2)	98.1 (93.1−103.2)
Untreated asthmatics (n = 9)					
FE_{NO} (ppb)	13.9** (12.2−19.1)	11.4** (7.6−17.1)	11.0* (7.5−15.9)	11.4* (8.1−16.0)	9.2 (5.8−15.0)
FEV_1 (%)	94.6 (87.8−101.4)	97.4 (88.9−106.0)	96.5 (87.9−105.0)	98.4 (88.4−108.3)	97.9 (89.6−106.4)
Treated asthmatics (n=8)					
FE_{NO} (ppb)	7.1 (5.3−9.5)	6.8 (4.8−9.7)	6.2 (4.6−8.4)	6.2 (4.4−9.0)	6.0 (4.5−8.1)
FEV_1 (%)	87.5* (78.7−96.4)	86.1* (76.4−95.9)	89.5* (84.9−94.2)	84.3* (74.2−94.5)	87.8* (78.8−96.8)

*$p < 0.05$ and **$p < 0.005$ vs healthy subjects by Kruskal-Wallis test.
FEV_1 is expressed as percent of predicted. Values are geometric (FE_{NO}) or arithmetic means (FEV_1) and 95% confidence intervals.

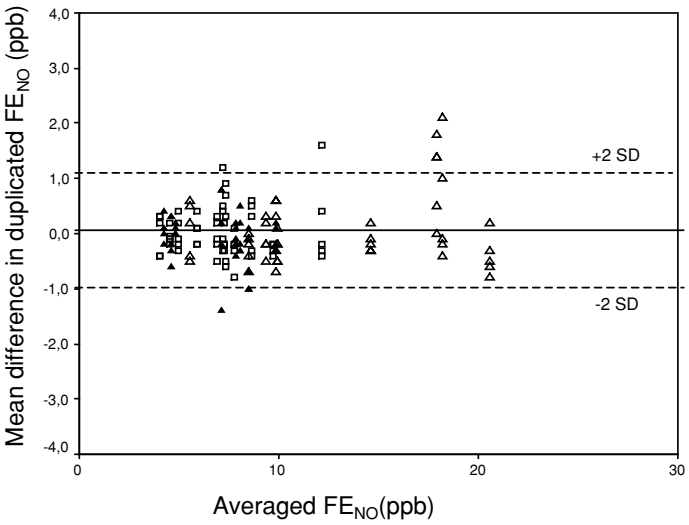

FIGURE 1.—Within-session differences for two exhaled nitric oxide (FE_{NO}) measurements in healthy (open squares), corticosteroid-naïve asthmatic children (open triangles), and steroid-treated asthmatic children (closed triangles). Note the highly repeatable FE_{NO} measurements in all groups.

apart and calculated ICC values between 0.70 and 0.89 (according to the group), in keeping with measurements reported by Stark et al. (31). These values are lower than those reported by Kharitonov et al. (ICC 0.99) who compared FE_{NO} levels from a shorter session-interval (morning versus afternoon) in healthy and asthmatic children (17). The similarity we found between morning and evening FE_{NO} measurements supports previous studies suggesting that this inflammatory marker undergoes scarce circadian variations (32, 33). A recent study nevertheless suggested that during winter time, FE_{NO} levels tend to be higher in the morning than in the evening (31).

Although our subjects showed high between-day FE_{NO} reproducibility, this tended to decrease after 6 days, mainly in the corticosteroid-naive asthmatic children (ICC 0.13). No data are available on FE_{NO} reproducibility 1 week-apart in subjects living in the same environment. An epidemiological study using a different FE_{NO} technique in young adults reported that differences between measurements 1 week apart deviated (CR = 2 SD) by 11 ppb, a CR about twice to four times higher than found in our healthy children and treated asthmatic children but twice lower than the CR we measured in our corticosteroid-naive asthmatics (34). These discrepancies most probably depend on the age groups studied, methods, and ambient characteristics in the two studies.

Despite the high FE_{NO} variation of first-versus-seventh morning found in our corticosteroid-naive subjects (CR 20.8 ppb), all of them remained under the risk level for asthma relapse (49 ppb) reported by Pijnenburg et al. (10). In this pediatric study, after corticosteroid withdrawal, 9 of the 40 children relapsed. In these children, geometric mean FE_{NO} values (expiratory flow of 50 mL/s) increased from 14.8 ppb to 35.3 ppb at 2 weeks and to 40.8 ppb at 4 weeks (versus 10.5, 15.7, and 15.9 ppb for the same intervals in those who did not relapse). Studying asthmatic adults, at an expiratory flow of 250 mL/s, Jones et al. found that a weekly variation in FE_{NO} levels over 10 ppb or 60% from baseline predicted loss of disease control after corticosteroid withdrawal (1). Low FE_{NO} levels and steadily low ambient NO concentrations could also explain why no subjects relapsed in our study. For instance, in a corticosteroid-naive child with a baseline FE_{NO} of 15.7 ppb the calculated weekly increase (128%) achieved 35.8 ppb, a value under the mentioned relapse-risk level of 49 ppb (10). We cannot exclude the possibility that FE_{NO} could increase further over 4 weeks and asthma symptoms could develop.

Our corticosteroid treated children had steadily low and highly reproducible FE_{NO} levels between sessions 1 week apart (ICC 0.88). Lower ICC values (0.52) have been reported in asthmatic children treated with inhaled budesonide between sessions 2 weeks apart (10). Values of 22 to 28 ppb have been reported as predictors of exacerbations of asthma in subjects under therapy with inhaled corticosteroids (11, 12). None of our corticosteroid-treated children reached these reported levels.

Unlike previous studies we explicitly chose to study inpatients rather than outpatients because we thought that staying in a similar ambient could reduce the potential effect of the ambient where the patients live. This precaution is especially important given the substantial changes in FE_{NO} measurements related to indoor allergens (e.g., house dust mites) and outdoor contaminants (ozone, particulate matter) (35–39).

Because FE_{NO} levels tended to decrease during the 1-week study period (especially after the first 12 hours) in untreated asthmatic children, we cannot exclude the possibility that these children were "challenged" by various ambient stimuli before arriving at the sanatorium. In contrast, corticosteroid–treated asthmatic children could be "protected" against the pro-inflammatory effect of these factors. Among these stimuli (whose effects respond to inhaled corticosteroids) are allergen exposure (40, 41) or ambient outdoor pollution (37, 38). The slightly decreasing FE_{NO} levels during the 1-week study period in untreated asthmatic children presumably affected their FE_{NO}reproducibility during this interval.

TABLE 3.—Within and between session differences for two exhaled nitric oxide (FE_{NO}) measurements.

Subjects	Within session	Between session		
		12 hours	24 hours	7 days
No. measurements	154	58	58	29
Healthy children (n = 12) MD (CR) ICC	0.01 (0.86) 0.99	0.17 (4.7) 0.80	−0.51 (4.8) 0.79	−1.14 (5.7) 0.65
Untreated asthmatics (n = 9) MD (CR) ICC	0.06 (1.30) 0.99	0.83 (9.8) 0.79	1.69 (9.6) 0.79	1.78 (20.8) 0.13
Treated asthmatics (n = 8) MD (CR) ICC	−0.08 (0.88) 0.99	0.03 (5.0) 0.70	0.74 (3.1) 0.89	0.94 (3.0) 0.88

Mean differences (MD) and coefficients of repeatability (CR = 2 SD) are expressed in ppb units.

ICC: intraclass correlation coefficient. Within session CR and ICC were calculated from all FE_{NO} measurements (5 sessions). Between session CR and ICC were calculated by multiplying the number of FE_{NO} measurements by the number of subjects as described in the Methods section.

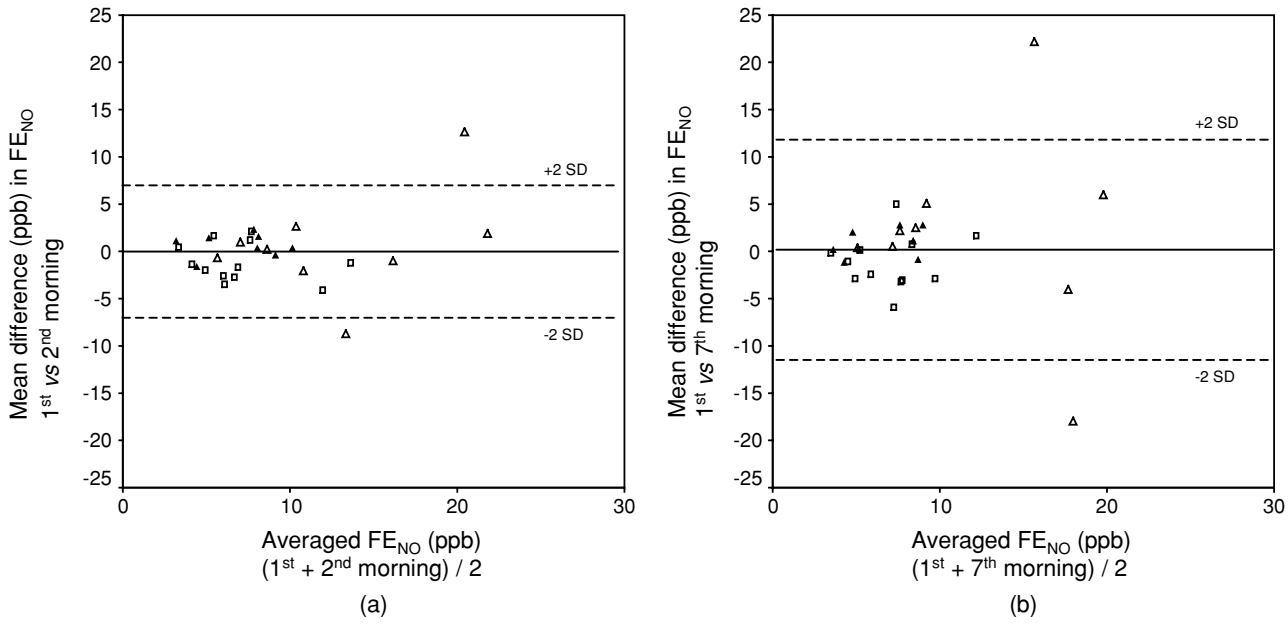

FIGURE 2.—Between-session differences for two mean exhaled nitric oxide (FE_{NO}) measurements in healthy (open squares), corticosteroid-naïve asthmatic children (open triangles), and steroid-treated asthmatic children (closed triangles). Note the wider between-session FE_{NO} variability for measurements on the 1st vs. 7th morning than on the 1st vs. 2nd morning, especially in corticosteroid-naive asthmatic subjects (open triangles, mean difference 1.8 ppb, 2 SD = 20.8 ppb).

To exclude a possible cause of bias that might have induced an opposite effect (increased rather than decreased FE_{NO}) we ascertained that sensitization to perennial and seasonal allergens was similar in both of our asthmatic groups (corticosteroid treated or not) during the 1-week stay. This effect is especially important for exposure to perennial allergens such as house dust mites, molds, and cat dander (14, 35) insofar as exposure to seasonal allergens leaves FE_{NO} levels unchanged (35). The possible influence of pollens therefore seems unlikely to account for FE_{NO} variations in sensitized-untreated asthmatic children during the season in which we undertook the study (summer time).

A possible limitation of our study is that its duration was too short to assess a relationship between FE_{NO}changes and respiratory symptoms. Indeed, none of the corticosteroid-naive asthmatic patients experienced symptoms requiring therapy despite their apparently large FE_{NO} variations nor did corticosteroid-naive patients require additional increases in their inhaled dose of corticosteroids. Longer periods are therefore required to assess FE_{NO} variations in asthmatic children with good disease control.

In conclusion, in healthy subjects and in asthmatic children receiving therapy with inhaled corticosteroids, FE_{NO} measurements are reproducible across a week. In corticosteroid-naive asthmatic children FE_{NO} levels are less reproducible. Possible long-term FE_{NO} variations (over weeks or months) in asthmatic children remain an interesting question for further research.

REFERENCES

1. Jones SL, Kittelson J, Cowan JO et al. The predictive value of exhaled nitric oxide measurements in assessing changes in asthma control. Am J Respir Crit Care Med 2001; 164:738–43.
2. Jatakanon A, Lim S, Barnes PJ. Changes in sputum eosinophils predict loss of asthma control. Am J Respir Crit Care Med 2000; 161:64–72.
3. Zacharasiewicz, Wilson N, Lex C et al. Clinical use of non-invasive measurements of airway inflammation in steroid reduction in children. Am J Respir Crit Care Med 2005; 171:1077–82.
4. Kharitonov SA, Yates DH, Chung KF, Barnes PJ. Changes in the dose of inhaled steroid affect exhaled nitric oxide levels in asthmatic patients. Eur Respir J 1996; 9:196–201.
5. Delgado-Corcoran C, Kissoon N, Murphy SP, Duckworth LJ. Exhaled nitric oxide reflects asthma severity and asthma control. Pediatr Crit Care Med 2004; 5:48–52.
6. Beck-Ripp J, Griese M, Arenz S, Köring C, Pasqualoni B, Bufler P. Changes of exhaled nitric oxide during steroid treatment of childhood asthma. Eur Respir J 2002; 19:1015–9.
7. Pijnenburg MW, Bakker EM, Hop WC, De Jongste JC. Titrating steroids on exhaled nitric oxide in children with asthma: a randomized controlled trial. Am J Respir Crit Care Med 2005; 172:831–6.
8. Robroeks CMHHT, van de Kant KDG, Jöbsis Q et al. Exhaled nitric oxide and biomarkers in exhaled breath condensate indicate the presence, severity and control of childhood asthma. Clin Exp Allergy 2007; 37:1303–11.
9. Pijnenburg MWH, Bakker EM, Lever S, Hop WC, De Jongste JC. High fractional concentration of nitric oxide in exhaled air despite steroid treatment in asthmatic children. Clin Exp Allergy 2005; 35:920–5.
10. Pijnenburg MW, Hofhuis W, Hop WC, De Jongste JC. Exhaled nitric oxide predicts asthma relapse in children with clinical asthma remission. Thorax 2005; 60:215–18.
11. Gelb ASF, Taylor CF, Shinar CM, Gutierrez C, Zamel N. Role of spirometry and exhaled nitric oxide to predict exacerbations in treated asthmatics. Chest 2006; 129:1492–9.
12. Fritsch M, Uxa S, Horak Jr F et al. Exhaled nitric oxide in the management of childhood asthma: A prospective 6-months study. Pediatr Pulmonol 2006; 41:855–62.
13. Zacharasiewicz A, Wilson N, Lex C, LI A, Bush A. Effect of inhalation times on exhaled NO. Pediatric Pulmonology 2004; 38:335–8.
14. Barreto M, Villa MP, Martella S et al. Exhaled nitric oxide in asthmatic and non-asthmatic children: Influence of type of allergen sensitization and exposure to tobacco smoke. Pediatr Allergy Immunol 2001; 12:247–56.
15. Paraskakis E, Brindicci C, Fleming L et al. Measurements of bronchial and alveolar nitric oxide production in normal children and children with asthma. Am J Crit Care Med 2006; 174:260–7.

16. Pedroletti C, Zetterquist W, Nordvall L, Alving K. Evaluation of exhaled nitric oxide in schoolchildren at different exhalation flow rates. Pediatr Res 2002; 52:393–8.
17. 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; 21:433–8.
18. Latzin P, Beck J, Griese M. Exhaled nitric oxide in healthy children: variability and a lack of correlation with atopy. Pediatr Allergy Immunol 2002; 13:37–46.
19. Buchvald F, Baraldi E, Carraro S et al. Measurements of exhaled nitric oxide in healthy subjects age 4 to 17 years. J Allergy Clin Immunol 2005; 115:1130–6.
20. Kissoon N, Duckworth LJ, Blake KY et al. Exhaled nitric oxide concentrations: online versus offline values in healthy children. Pediatr Pulmonol 2002; 33:283–92.
21. American Thoracic Society Documents. 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; 171:912–30.
22. Barreto M, Villa MP, Montesano M et al. Reduced exhaled nitric oxide in children after testing of maximal expiratory pressures. Pediatr Pulmonol 2006; 41:141–5.
23. Nathan RA, Sorkness CA, Kosinski M et al. Development of the asthma control test: A survey for assessing asthma control. J Allergy Clin Immunol 2004; 113:59–65.
24. American Thoracic Society. Medical Section of American Lung Association. Standardization of spirometry: 1994 update. Am J Respir Crit Care Med 1995; 153:1107–36.
25. Quanjer PH, Stocks J, Polgar G, Wise M, Karlberg J, Borsboom G. Compilation of reference values for lung function measurements in children. Eur Respir J Suppl 1989; 4:184s–261s.
26. Barreto M, Villa MP, Martella S et al. Off-line exhaled nitric oxide measurements in children. Pediatr Pulmonol 2001; 32:159–67.
27. Silkoff PE, McClean PA, Slutsky AS et al. Marked flow-dependence of exhaled nitric oxide using a new technique to exclude nasal nitric oxide. Am J Respir Crit Care Med 1997; 155:260–7.
28. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986; 1:307–10.
29. Bland JM, Altman DG. Measurement error and correlation coefficients. Br Med J 1996; 313:41–2.
30. Faul JL, Demers EA, Burke CM, Poulter LW. The reproducibility of repeat measures of airway inflammation in stable atopic asthma. Am J Respir Crit Care Med 1999; 160:1457–61.
31. Stark H, Purokivi M, Kiviranta J, Randell J, Tukiainen H. Short-term and seasonal variations of exhaled and nasal NO in healthy subjects. Respir Med 2006; 21:1–7.
32. Georges G, Bartelson BB, Martin RJ, Silkoff PE. Circadian variation in exhaled nitric oxide in nocturnal asthma. J Asthma 1999; 36:467–73.
33. ten Hacken NH, van der Vaart H, van der Mark TW, Koeter GH, Postma DS. Exhaled nitric oxide is higher both at day and night in subjects with nocturnal asthma. Am J Respir Crit Care Med 1998; 158:902–7.
34. Salome CM, Roberts AM, Brown NJ, Dermand J, Marks GB, Woolcock AJ. Exhaled nitric oxide measurements in a population sample of young adults. Am J Respir Crit Care Med 1999; 159:911–6.
35. Leuppi JD, Downs SH, Downie SR, Marks GB, Salome CM. Exhaled nitric oxide levels in atopic children: relation to specific allergen sensitisation, AHR, and respiratory symptoms. Thorax 2002; 57:518–23.
36. Olin A-C, 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.
37. Koeing JQ, Jansen K, Mar TF et al. Measurement of offline exhaled nitric oxide in a study of community exposure to air pollution. Environ Health Perspect 2003; 111:1625–9.
38. Koeing JQ, Mar TF, Allen RW et al. Pulmonary effects of indoor and outdoor-generated particles in children with asthma. Environ Health Perspect 2005; 113:499–503.
39. Piacentini GL, Bodini A, Costella S et al. Allergen avoidance is associated with a fall in exhaled nitric oxide in asthmatic children. J Allergy Clin Immunol 1999; 104:1323–4.
40. Prieto L, Gutierrez V, Perez-Frances C et al. Effect of fluticasone propionate-salmeterol therapy on seasonal changes in airway responsiveness and exhaled nitric oxide levels in patients with pollen-induced asthma. Ann Allergy Asthma Immunol 2005; 95:452–61.
41. De Kluijver J, Evertse CE, Schrumpf JA et al. Asymptomatic worsening of airway inflammation during low-dose allergen exposure in asthma: protection by inhaled steroids. Am J Respir Crit Care Med 2002; 166:294–300.