

# Exhaled carbon monoxide levels after a course of oral prednisone in children with asthma exacerbation

Stefania Zanconato, MD, PhD, Massimo Scollo, MD, Cristina Zaramella, MD,  
Linda Landi, MD, Franco Zacchello, MD, and Eugenio Baraldi, MD *Padova, Italy*

Asthma, rhinitis,  
other respiratory  
diseases

**Background:** Fractional exhaled nitric oxide ( $FE_{NO}$ ) and exhaled carbon monoxide (ECO) have been proposed as markers of airway inflammation and oxidative stress.

**Objective:** The aim of this study was to assess the effect of oral prednisone treatment on  $FE_{NO}$  and ECO levels in a group of 30 asthmatic children with asthma exacerbation.

**Methods:** Thirty asthmatic children with asthma exacerbation were treated with oral prednisone for 5 days (1 mg/kg/day). Before and after prednisone therapy, ECO was measured by means of a chemical analyzer and  $FE_{NO}$  was measured by means of a chemiluminescence analyzer. ECO and  $FE_{NO}$  were also measured in a group of healthy nonatopic children.

**Results:** Before therapy, both ECO values and  $FE_{NO}$  values were higher in asthmatic children (ECO,  $3.2 \pm 0.2$  ppm;  $FE_{NO}$  online,  $74.9 \pm 6.2$  ppb;  $FE_{NO}$  offline,  $20.2 \pm 1.4$  ppb) than in healthy controls (ECO,  $2.0 \pm 0.2$  ppm [ $P < .01$ ];  $FE_{NO}$  online,  $10.1 \pm 0.8$  [ $P < .0001$ ];  $FE_{NO}$  offline,  $5.9 \pm 0.4$  ppb [ $P < .0001$ ]). An overlap in ECO values was found between healthy controls and asthmatic children. After prednisone therapy, there was a significant reduction in  $FE_{NO}$  values ( $FE_{NO}$  online,  $40.6 \pm 4.6$  ppb [ $P < .0001$ ];  $FE_{NO}$  offline,  $11.1 \pm 0.8$  ppb [ $P < 0.0001$ ]) and a slight but nonsignificant decrease in ECO values ( $2.7 \pm 0.2$  ppm [ $P =$  not significant]) in the asthmatic group. No significant correlation between ECO values and  $FE_{NO}$  values was found in either the asthmatic children or the controls.

**Conclusions:** After a course of prednisone therapy, in children with asthma exacerbation there is a significant decrease in  $FE_{NO}$  but no significant change in ECO levels. This possibly suggests that ECO is less sensitive than  $FE_{NO}$  to inhibition by corticosteroids. (J Allergy Clin Immunol 2002;109:440-5.)

**Key words:** Asthma, exhaled carbon monoxide, exhaled nitric oxide, children, glucocorticoids

The chronic inflammation and oxidative stress<sup>1</sup> that characterize asthma and other lung diseases are not measured directly in routine clinical practice. There is increasing interest in the use of exhaled markers as a non-invasive means of detecting and monitoring airway

## Abbreviations used

ECO: Exhaled carbon monoxide  
 $FE_{NO}$ : Fractional exhaled nitric oxide  
HO: Heme oxygenase  
NOS: Nitric oxide synthases

inflammation and assessing anti-inflammatory treatment. Fractional exhaled nitric oxide ( $FE_{NO}$ ), the most extensively studied exhaled marker, appears to increase in patients with asthma and might be related to asthma control.<sup>1,2</sup> Nitric oxide (NO) is generated from L-arginine by the NO synthases (NOS) found in many cells of the lung, including pulmonary nerves, airway epithelial cells, pulmonary vascular endothelial cells, and alveolar macrophages.<sup>3</sup> The constitutive isoform of NOS seems to release NO in physiologic conditions, whereas it has been suggested that the elevated exhaled NO levels found in asthmatic subjects might be due to an increase in expression of inducible NOS in the respiratory tract induced by the action of proinflammatory cytokines.<sup>4</sup> Several studies have shown a reduction in exhaled NO after glucocorticoid treatment and have suggested that NO might be used as a marker of airway inflammation in asthma.<sup>1,5,6</sup>

Elevated levels of carbon monoxide (CO) have recently been shown in the exhaled air of adult patients with asthma.<sup>7-9</sup> CO is endogenously produced by the class of enzymes known as heme oxygenase (HO).<sup>10</sup> Two isoforms of HO have been described: the constitutive HO-2 and the inducible HO-1. HO-1, an important component of the protective response to oxidative stress, is activated by a variety of proinflammatory cytokines,<sup>11</sup> NO,<sup>12</sup> and oxidants.<sup>13</sup> Therefore, high concentrations of exhaled CO (ECO) have been suggested to reflect a pathway of airway inflammation in asthmatic patients.<sup>1,8,9</sup> At this time, little is known about ECO in children. A recent study<sup>14</sup> has shown that children with persistent asthma have higher ECO levels than healthy children. To our knowledge, no data are available on ECO levels in children with asthma exacerbation.

The aim of this study was to assess the effect of a course of oral glucocorticoids on  $FE_{NO}$  and ECO in children with asthma exacerbation. For this purpose, we measured  $FE_{NO}$  and ECO before and after 5 days of oral prednisone treatment in children with asthma exacerbation.

From the Department of Pediatrics, Unit of Respiratory Medicine and Allergy, University of Padova.

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Reprint requests: Eugenio Baraldi, MD, Department of Pediatrics, Via Giustiniani 3, 35128 Padova, Italy.

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## METHODS

### Study subjects

**Asthmatic children.** The study included 30 asthmatic nonsmoking children (19 male and 11 female) with acute asthma exacerbation. Their mean age was  $10.5 \pm 0.5$  years (range, 4–15 years), and all were atopic and sensitized to common allergens (mixed grass pollen, *Parietaria* species, *Artemisia vulgaris*, *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, *Alternaria* species, and cat), as demonstrated by skin prick test and/or RAST results. They were recruited from patients attending the Pulmonology/Allergy outpatient clinic at the Department of Pediatrics of Padova.

In accord with international guidelines,<sup>15</sup> the diagnosis of asthma was based on clinical history and examination, pulmonary function parameters, and pulmonary function response to inhaled  $\beta_2$ -agonist agents. Nineteen (63%) of the children had persistent asthma and had been on long-term treatment with low doses of inhaled steroids<sup>15</sup> for at least 1 month; 7 children (23%) were also on treatment with long-acting  $\beta_2$  agonists. The other 11 children had intermittent asthma and were on no long-term treatment. All children used inhaled short-acting  $\beta_2$  agonists on demand. Exclusion criteria included the following: children presenting wheezing for the first time; use of oral or parenteral corticosteroids in the last month before the study; body temperature greater than 38°C; concurrent cardiopulmonary, hepatic, and renal diseases.

Asthma exacerbation was defined as increased asthma signs and symptoms (coughing, wheezing, shortness of breath) unresponsive to the patient's routine asthma medication and additional  $\beta_2$ -agonist therapy. The children were examined by a physician and underwent  $FE_{NO}$  and ECO measurements and spirometry. Oral prednisone treatment (1 mg/kg/day) was then started in all patients. After 5 days of therapy, the patients were re-examined and  $FE_{NO}$  and ECO measurements and spirometry were repeated.

**Healthy control subjects.** We studied 29 healthy, nonsmoking children (14 male and 15 female; mean age,  $8.5 \pm 0.5$  years [range, 5–13 years]) with negative skin prick test results and normal pulmonary function parameters. None had any history of allergy, respiratory disease, or recent respiratory tract infections.

The study was approved by the Ethics Committee of our Department, and informed consent was obtained from the parents of all subjects.

### ECO measurement

ECO was measured in 30 asthmatic patients and 21 healthy children. The measurement of CO in the exhaled air was performed through use of a modified electrochemical analyzer (Crowcon TX, Oxfordshire, United Kingdom) sensitive to CO within the range of 0 to 100 parts per million (ppm) and with a resolution of 0.1 ppm. Before each study the analyzer was checked with a certified calibration mixture (10 ppm) of CO (SIAD, Bergamo, Italy) with guaranteed stability. After full exhalation, the subject was asked to inhale deeply through the mouth and hold the breath for 15 seconds. The subject was then instructed to exhale into a mouthpiece directly connected to a bag collection device with discharge of the dead space (150 mL for children 4–8 years old and 200 mL for children 9–15 years old), as previously described for  $FE_{NO}$  measurement.<sup>16</sup> This procedure was repeated after a 2-minute normal breathing interval; the mean value was used for analysis. The reservoir was immediately connected to the CO analyzer, which had a sampling flow rate of 1 L/min. The CO level in the ambient air was measured before each maneuver and was subtracted from the mean value obtained from the 2 measurements. Ambient CO levels were always less than 1.6 ppm.

To assess the influence of airway caliber on ECO levels, we measured CO before and after bronchodilator challenge in another

group of 16 stable asthmatic children attending our clinic (10 male and 6 female; mean age,  $11 \pm 0.6$  years [range, 9–14 years]). For this test, the subject was asked to inhale 300  $\mu$ g of albuterol by metered dose inhaler with a spacer device. Each of the children had significant reversibility, as indicated by a  $FEV_1$  increase of  $\geq 12\%$ .

### FENO measurement

**Offline method.** In 29 asthmatic and 23 healthy subjects,  $FE_{NO}$  measurement was performed through use of an offline method with discharge of the dead space (150 mL for children 4–8 years old and 200 mL for children 9–15 years old), as previously described.<sup>16</sup> Each subject was asked to orally inhale to total lung capacity and to exhale through a restrictor connected to a T-valve that allowed the separation of exhaled air into a first portion (dead space balloon) that was discarded and a remaining portion that was collected into a Mylar reservoir (Morgan Medical, Kent, United Kingdom). To eliminate contamination by ambient NO, the subject breathed for 30 seconds from a reservoir filled with NO-free air. Gas samples were analyzed immediately.

**Online method.** In 18 asthmatic and 22 healthy subjects,  $FE_{NO}$  was measured through use of an online method by means of a computerized system (Exhaled Breath Analyzer, Aerocrine AB, Stockholm, Sweden) with a chemiluminescence analyzer (CLD 77 AM, Eco Physics, Duernten, Switzerland) according to the American Thoracic Society's recommendations,<sup>17</sup> as previously described.<sup>18</sup> The subject inhaled NO-free air through the mouth and exhaled with a target flow of 50 mL/s against a resistor for a minimum of 6 to 7 seconds until an NO plateau of at least 2 seconds was achieved.

### Spirometry

Pulmonary function parameters ( $FEV_1$ , forced vital capacity [FVC], and forced expiratory flow at 25% to 75% of forced vital capacity [ $FEF_{25-75}$ ]) were measured by means of a 10-L bell spirometer (Biomedin, Padova, Italy); the best of 3 maneuvers was expressed as a percentage of predicted reference values, according to the method of Polgar and Promadhat.<sup>19</sup> Spirometry was performed after  $FE_{NO}$  and ECO measurements.

### Statistical analysis

Results are expressed as means  $\pm$  SEMs. A computer package (Statistica, Microsoft, Redmond, Wash) was used for statistical analysis. Correlation between  $FE_{NO}$  and ECO levels and pulmonary function parameters was evaluated through use of Spearman rank correlation testing.  $FE_{NO}$  and ECO levels and pulmonary function parameters in asthmatic children before and after 5 days of prednisone therapy were compared through use of Wilcoxon matched-paired testing. Comparisons of  $FE_{NO}$  and ECO levels between asthmatic and healthy subjects and of measured  $FE_{NO}$  values between the online and offline methods were made through use of Mann-Whitney testing. ECO levels before and after the bronchodilation test were compared through use of Wilcoxon matched-paired testing. Results were considered significant at a  $P$  value of  $< .05$ .

## RESULTS

$FE_{NO}$  and ECO levels were significantly higher in children with acute asthma exacerbation before steroid treatment than in healthy subjects ( $FE_{NO}$  online: asthmatic,  $74.9 \pm 6.2$  ppb, control  $10.1 \pm 0.8$  ppb [ $P < .0001$ ];  $FE_{NO}$  offline: asthmatic  $20.2 \pm 1.4$  ppb, control  $5.9 \pm 0.4$  ppb [ $P < .0001$ ]; ECO: asthmatic  $3.2 \pm 0.2$  ppm, control,  $2.0 \pm 0.2$  ppm [ $P < .01$ ]; Fig 1).

In the asthmatic children,  $FE_{NO}$  levels decreased significantly after 5 days of oral prednisone therapy (online

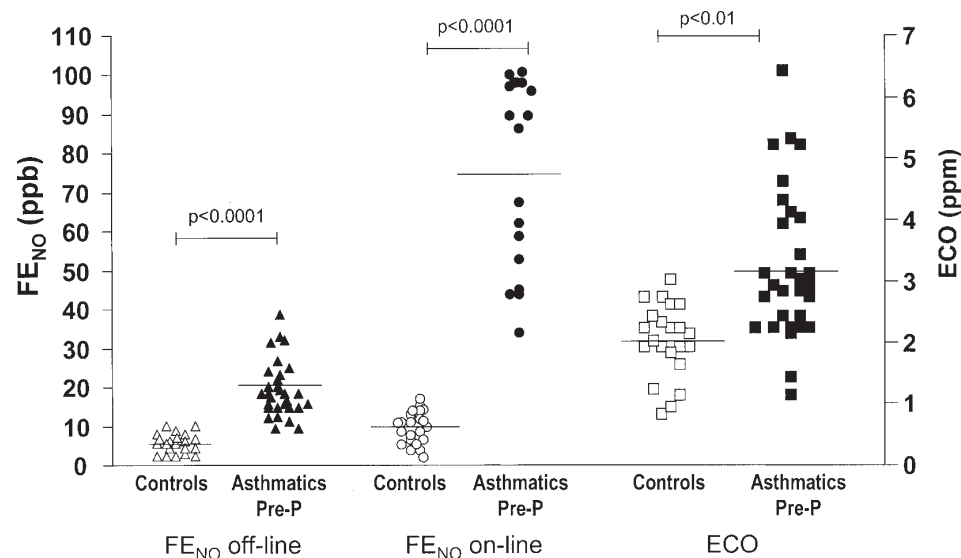


FIG 1.  $FE_{NO}$  and ECO values in healthy subjects and before prednisone treatment (P) in children with acute asthma exacerbation. Individual and mean values are shown.

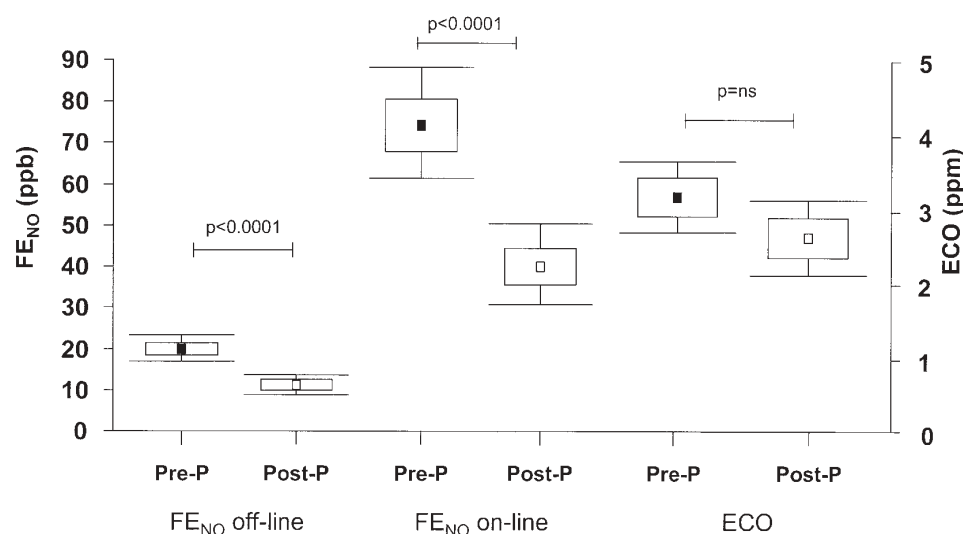


FIG 2.  $FE_{NO}$  and ECO values measured in asthmatic children before and after 5 days of prednisone therapy (P). Mean, SEM, and CI values are shown.

method:  $40.6 \pm 4.6$  ppb [ $P < .0001$ ]; offline method:  $11.1 \pm 0.8$  ppb [ $P < .0001$ ], whereas there was no significant change in ECO values ( $2.7 \pm 0.2$  ppm [ $P = \text{not significant}$ ]; Fig 2). After prednisone treatment,  $FE_{NO}$  levels in the asthmatic children were still higher than those in the controls (online,  $P < .0001$ ; offline,  $P < .0001$ ), whereas no significant difference was found in ECO values ( $P = \text{not significant}$ ) between asthmatic children and healthy children.

We compared the ECO and  $FE_{NO}$  values of the asthmatic children on long-term inhaled steroid treatment with those of steroid-naïve subjects. Before prednisone treatment, ECO values were higher in steroid-treated children ( $3.5 \pm 0.3$  ppm [ $P < .05$ ]), whereas  $FE_{NO}$  values were

similar in the 2 groups (online: steroid-treated  $74.8 \pm 10.7$  ppb, steroid-naïve,  $74.9 \pm 7.9$  [ $P = \text{not significant}$ ]; offline: steroid-treated  $19.3 \pm 1.9$  ppb, steroid-naïve  $21.8 \pm 2.2$  [ $P = \text{not significant}$ ]). After prednisone treatment, ECO values did not significantly change in either group (steroid-treated,  $2.7 \pm 0.3$  ppm [ $P = \text{not significant}$ ]; steroid-naïve,  $2.7 \pm 0.5$  [ $P = \text{not significant}$ ]), whereas  $FE_{NO}$  values decreased significantly in both groups (online: steroid-treated  $38.2 \pm 6.4$  ppb [ $P < .01$ ], steroid-naïve  $43.1 \pm 6.9$  [ $P < .01$ ]; offline: steroid-treated  $11.8 \pm 1.2$  ppb [ $P < .001$ ], steroid-naïve  $9.8 \pm 0.9$  [ $P < .01$ ]).

We found a significant correlation between  $FE_{NO}$  measured with the offline technique and  $FE_{NO}$  measured with the online technique ( $r = 0.85$ ;  $P < .0001$ ). We obtained

**TABLE I.** ECO and FE<sub>NO</sub> values measured in asthmatic children (before and after 5 days of prednisone therapy) and healthy children

|                    | FE <sub>NO</sub> offline (ppb) |                          | FE <sub>NO</sub> online (ppb) |                           | ECO (ppm)              |                        |
|--------------------|--------------------------------|--------------------------|-------------------------------|---------------------------|------------------------|------------------------|
|                    | Before                         | After                    | Before                        | After                     | Before                 | After                  |
| Asthmatic children | 20.2 ± 1.4<br>(17.2-23.3)      | 11.1 ± 0.8<br>(9.3-12.8) | 74.9 ± 6.2<br>(61.8-88.0)     | 40.6 ± 4.6<br>(30.9-50.4) | 3.2 ± 0.2<br>(2.7-3.7) | 2.7 ± 0.2<br>(2.2-3.2) |
| Control children   | 5.9 ± 0.4<br>(5.0-6.9)         |                          | 10.1 ± 0.8<br>(8.2-11.8)      |                           | 2.0 ± 0.2<br>(1.7-2.3) |                        |
| <i>P</i> values    | <.0001                         | <.0001                   | <.0001                        | <.0001                    | <.01                   | NS                     |

Data are expressed as means ± SEMs; 95% CIs are given in parentheses.

FE<sub>NO</sub>, Fractional exhaled nitric oxide; ECO, exhaled carbon monoxide; NS, Not significant.

higher FE<sub>NO</sub> values with the online technique than with the offline technique ( $P < .0001$  before and after therapy in asthmatic children and  $P < .0005$  in healthy children). The ECO and FE<sub>NO</sub> 95% CIs are shown in Table I. No significant correlation between ECO and FE<sub>NO</sub> values ( $P =$  not significant) was found in either the asthmatic group or the control group.

In the asthmatic children, after 5 days of prednisone, a significant improvement was found in pulmonary function parameters (FEV<sub>1</sub>, from 68% of predicted values to 88% [ $P < .0005$ ]; FEF<sub>25-75</sub>, from 42% to 66% [ $P < .0005$ ]; FVC, from 83% to 98% [ $P < .001$ ]). Neither ECO nor FE<sub>NO</sub>, as measured with the online and offline methods, was significantly correlated with any of the pulmonary function test parameters (FVC, FEV<sub>1</sub>, FEF<sub>25-75</sub>;  $P =$  not significant). Similarly, after prednisone treatment, the changes in ECO and FE<sub>NO</sub> were not correlated with the changes in pulmonary function parameters ( $P =$  not significant).

In the 16 stable asthmatic children who underwent bronchodilation testing, there was an increase of 17% ± 3% in FEV<sub>1</sub>, whereas no changes were found in ECO levels (baseline, 2.5 ppm; after bronchodilation, 2.1 ppm [ $P =$  not significant]).

## DISCUSSION

Our study shows that children with acute asthma exacerbation have a modest increase in ECO and a consistent increase in FE<sub>NO</sub> values in comparison with normal controls. After a 5-day course of oral prednisone, there was a significant reduction in FE<sub>NO</sub> and a slight but non-significant decrease in ECO levels. There is no correlation between ECO level and FE<sub>NO</sub> level, and neither is correlated with pulmonary function parameters.

It has been suggested that detection of ECO, as an index of HO-1 activity, might reflect oxidative stress and could be a simple, noninvasive tool for monitoring airway inflammation.<sup>1,8,9</sup> There is some evidence that ECO levels in asthmatic patients are increased in comparison with those in healthy nonsmoking subjects<sup>7,9</sup> and that they could be related to the severity of asthma.<sup>20</sup>

In children, Uasuf et al<sup>14</sup> have shown that ECO levels are significantly higher in patients with persistent asthma on inhaled steroid treatment than in patients with infrequent episodic asthma and healthy controls,<sup>14</sup> even though there was an overlap in ECO levels between con-

trol and asthmatic subjects. An overlap in ECO levels between healthy children and asthmatic children was also found in our study, and the difference in ECO values between the 2 groups was much less than the difference in FE<sub>NO</sub> values.

Even though our data were collected during acute asthma exacerbation, children with persistent asthma on inhaled steroid therapy had higher ECO values, in accord with the findings of Uasuf et al,<sup>14</sup> than children with intermittent asthma not treated with inhaled steroids, whereas FE<sub>NO</sub> values were similar in the 2 groups. This suggests that ECO might be related to the severity of asthma and oxidative stress and might be less sensitive to inhaled steroid inhibition than FE<sub>NO</sub>. After prednisone therapy, FE<sub>NO</sub> levels were decreased both in children on inhaled steroid therapy and in the steroid-naïve group, whereas ECO values were slightly, but not significantly, decreased only in the inhaled steroid-treated group. The interpretation of our results could be limited by the relatively small number of patients included in the 2 groups, and we cannot exclude the possibility that with a larger sample of subjects the difference could be significant.

In a recent study by Lim et al,<sup>21</sup> after 1 month of treatment with inhaled budesonide, there was no significant change in the expression and distribution of both HO-1 and HO-2 in the airway submucosa of patients with mild asthma despite a significant reduction in airway eosinophils and a reduction in bronchial responsiveness to methacholine. Levels of FE<sub>NO</sub> were significantly reduced after budesonide treatment, but exhaled ECO levels remained unchanged, suggesting that corticosteroids might not be very effective in reducing oxidative stress in mild asthma.

Yamaya et al<sup>22</sup> reported that in adults with acute asthma exacerbation and symptoms of upper respiratory tract infection, oral glucocorticoid treatment significantly decreased ECO concentrations. All patients had persistent asthma and were on long-term inhaled steroid treatment, whereas 11 of our patients had intermittent asthma. In addition, the steroid treatment period was longer in the study of Yamaya et al than in our investigation, and in at least 8 of their 20 subjects ECO levels decreased only after 14 days of therapy, whereas in our study ECO was measured only after 5 days of prednisone treatment. Finally, all patients included in the study of Yamaya et al had symptoms of upper respiratory tract infection, and it

is known that viral infections can induce HO in a variety of cell types, including airway epithelial cells and macrophages,<sup>23</sup> via the induction of proinflammatory cytokines<sup>11</sup> and NO.<sup>12</sup>

Thus the number of investigations of asthma and ECO is still limited, and the results are not consistent. The elevated ECO observed might suggest a role for oxidative stress in asthma, but the role of ECO measurement in the assessment of asthmatic inflammation has not been established. Furthermore, sensitivity of this marker might not be high enough to have clinical utility.

A great deal of work is available on exhaled NO measurements, which appear to have a potential role in the monitoring of asthma.<sup>1</sup> Elevated levels of NO have been shown to be associated with conditions known to increase inflammation in asthma, such as exposure to allergens.<sup>24,25</sup> Whereas ECO has been demonstrated to increase during both early and late asthmatic responses to allergen challenge, FE<sub>NO</sub> increases only during the late asthmatic response in association with inducible NOS activation, eosinophil infiltration, and plasma exudation.<sup>26</sup>

FE<sub>NO</sub> has been used to monitor anti-inflammatory treatment with inhaled corticosteroids in asthma and appears to be a sensitive and rapid marker of the effect of steroid treatment.<sup>1,6</sup> The assumption that FE<sub>NO</sub> reflects airway inflammation is supported by studies correlating FE<sub>NO</sub> levels with other markers of airway inflammation.<sup>1</sup> Several studies have shown a correlation between FE<sub>NO</sub> level and sputum eosinophil count in asthmatic children.<sup>27,28</sup>

In accord with the results of the present study, we reported in a previous article a significant decrease in FE<sub>NO</sub> in children with acute asthma exacerbation after 5 days of prednisone therapy.<sup>29</sup> We did not find a correlation between decrease in FE<sub>NO</sub> level and improvement in pulmonary function parameters in either study. It is known that FE<sub>NO</sub> levels can remain elevated in patients whose spirometry has normalized; this provides evidence for the presence of airway inflammation in patients with quiescent asthma.<sup>2</sup>

Formal recommendations for FE<sub>NO</sub> measurement have been published,<sup>17</sup> whereas a consensus on the proper technique for ECO measurement in children has not yet been established. FE<sub>NO</sub> is a flow-dependent parameter, low flows resulting in higher FE<sub>NO</sub> levels and vice versa.<sup>17</sup> In the present study, FE<sub>NO</sub> values obtained with the online method were well correlated with the values obtained with the offline method, but the latter measures were lower. This discrepancy can be explained by the higher flows used with the offline method. Jobsis et al<sup>30</sup> obtained very similar FE<sub>NO</sub> values with offline and online methods using the same expiratory flow rate. We observed that the online method with a flow rate of 50 mL/s resulted in a clear discrimination between asthmatic and healthy subjects without overlap between individual FE<sub>NO</sub> values.

For ECO measurement, we used the same bag collection method used for offline FE<sub>NO</sub>, with discharge of the dead space to ensure that measurements were performed in the same exhaled air compartment. Airway caliber is among the physiologic factors that have been suggested to interfere with the interpretation of exhaled gas mea-

surements in asthma. To assess whether airway caliber could affect ECO levels, we measured ECO before and after acute bronchodilation in a group of asthmatic children. Our findings, consistent with other recent data,<sup>22</sup> were that there were no changes in ECO levels when airway caliber was increased by albuterol administration.

In conclusion, after a course of oral prednisone therapy in a group of children with asthma exacerbation, there was a significant reduction in FE<sub>NO</sub> and a slight but nonsignificant reduction in ECO levels. Even though our study included a relatively small number of subjects, the results suggest that ECO might be less sensitive to corticosteroid inhibition than FE<sub>NO</sub>. More studies are required to define the biological role of CO in asthmatic airway inflammation and the utility of its assessment in clinical practice.

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