

Effects of inhaled corticosteroids on exhaled leukotrienes and prostanoids in asthmatic children

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Background: Lipid mediators play an important pathophysiologic role in atopic asthmatic children, but their role in the airways of atopic nonasthmatic children is unknown.

Objective: We sought (1) to measure leukotriene (LT) E₄, LTB₄, 8-isoprostane, prostaglandin E₂, and thromboxane B₂ concentrations in exhaled breath condensate in atopic asthmatic and atopic nonasthmatic children; (2) to measure exhaled nitric oxide (NO) as an independent marker of airway inflammation; and (3) to study the effect of inhaled corticosteroids on exhaled eicosanoids.

Methods: Twenty healthy children, 20 atopic nonasthmatic children, 30 steroid-naive atopic asthmatic children, and 25 atopic asthmatic children receiving inhaled corticosteroids were included in a cross-sectional study. An open-label study with inhaled fluticasone (100 µg twice a day for 4 weeks) was undertaken in 14 steroid-naive atopic asthmatic children.

Results: Compared with control subjects, exhaled LTE₄ ($P < .001$), LTB₄ ($P < .001$), and 8-isoprostane ($P < .001$) levels were increased in both steroid-naive and steroid-treated atopic asthmatic children but not in atopic nonasthmatic children (LTE₄, $P = .14$; LTB₄, $P = .23$; and 8-isoprostane, $P = .52$). Exhaled NO levels were increased in steroid-naive atopic asthmatic children ($P < .001$) and, to a lesser extent, in atopic nonasthmatic children ($P < .01$). Inhaled fluticasone reduced exhaled NO (53%, $P < .0001$) and, to a lesser extent, LTE₄ (18%, $P < .01$) levels but not LTB₄, prostaglandin E₂, or 8-isoprostane levels in steroid-naive asthmatic children.

Conclusions: Exhaled LTE₄, LTB₄, and 8-isoprostane levels are increased in atopic asthmatic children but not in atopic nonasthmatic children. In contrast to exhaled NO, these

markers seem to be relatively resistant to inhaled corticosteroids. (J Allergy Clin Immunol 2004;114:761-7.)

Key words: Leukotrienes, prostaglandins, exhaled breath condensate, exhaled nitric oxide, childhood asthma, airway inflammation, noninvasive markers, corticosteroids

Lipid mediators, including leukotrienes (LTs) and prostanoids, play an important pathophysiologic role in atopic children with asthma,¹ but their role in the airways of atopic nonasthmatic children is largely unknown. This is primarily due to the lack of noninvasive *in vivo* techniques for quantifying the levels of these inflammatory mediators in the respiratory system. Measurement of LTs and prostanoids in exhaled breath condensate (EBC) is an *in vivo* approach for studying the role of eicosanoids in asthma.^{2,3} EBC is a completely noninvasive method for the collection of airway secretions.^{4,5} By using immunoassays, cysteinyl leukotrienes (CysLTs), LTB₄, and 8-isoprostane, a marker of oxidative stress, were detected in EBC in healthy children, and levels of these were found to be increased in children with stable asthma.⁶⁻⁸ Exhaled CysLT and 8-isoprostane levels are also increased during asthma exacerbations.⁹ We have recently confirmed the presence of LTD₄, LTE₄, and LTB₄ in EBC from healthy children by using gas chromatography-mass spectrometry.¹⁰ Compared with those in healthy children, LT concentrations in EBC were increased in steroid-naive asthmatic children.¹⁰ However, whether atopy itself is responsible for increased levels of these inflammatory markers in atopic asthmatic children is not known because a group of atopic nonasthmatic children was not included in previous studies.^{6-8,10} Exhaled nitric oxide (NO) is a well-established marker of airway inflammation in asthmatic patients who are steroid naive.¹¹ Exhaled NO levels are generally higher in atopic compared with nonatopic groups, although to a lesser extent than those in atopic patients with asthma.¹²

In contrast to exhaled NO levels, which are sensitive to inhaled corticosteroids, the effect of these drugs on LT and prostanoid concentrations in EBC in childhood asthma is controversial. Establishing the effect of inhaled corticosteroids on exhaled LTs and prostanoids in asthmatic children is relevant for future studies aimed at using these markers in asthma management.

The aim of the present study was (1) to identify the profile of LTs and prostanoids in EBC in atopic asthmatic children and in atopic nonasthmatic children by measuring

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TABLE I. Subject characteristics*

	Healthy children	Atopic nonasthmatic children	Atopic steroid-naïve asthmatic children	Atopic steroid-treated asthmatic children
n	20	20	30	25
Age, y	9 ± 0.5	10 ± 0.8	9 ± 0.7	10 ± 0.6
Sex, F/M	10/10	11/9	14/16	12/13
FEV ₁ , % predicted	99.8 ± 2.7	100.3 ± 2.5	95.1 ± 1.8	95.8 ± 3.7
FVC, % predicted	100.4 ± 2.9	102.8 ± 2.9	98.2 ± 3.3	100.7 ± 2.2
FEV ₁ /FVC, %	97.7 ± 2.8	96.3 ± 2.4	84.2 ± 3.1†	83.1 ± 2.5†
FEF _{25%-75%}	102.0 ± 5.5	100.5 ± 4.9	70.2 ± 4.8‡	84.3 ± 5.0‡

FVC, Forced vital capacity.

*Data are expressed as n or mean ± SEM.

†*P* < .05 and ‡*P* < .01 compared with healthy children. There was not a statistical difference in FEF_{25%-75%} between steroid-naïve asthmatic children and steroid-treated asthmatic children.*Abbreviations used*

- CysLT: Cysteinyl leukotriene
 EBC: Exhaled breath condensate
 FEF_{25%-75%}: Forced expiratory flow between 25% and 75% of the vital capacity
 LT: Leukotriene
 NO: Nitric oxide
 PGE₂: Prostaglandin E₂
 RP-HPLC: Reverse-phase HPLC
 TxB₂: Thromboxane B₂

LTE₄, LTB₄, 8-isoprostane, prostaglandin (PG) E₂, and thromboxane B₂ (TxB₂) concentrations; (2) to measure exhaled NO as an independent marker of airway inflammation; and (3) to investigate the effect of inhaled corticosteroids on exhaled LTs and prostanoids in steroid-naïve atopic asthmatic children.

METHODS**Study subjects**

Four groups of white children were studied: 20 healthy nonatopic nonasthmatic children, 20 atopic nonasthmatic children, 30 steroid-naïve atopic children with stable mild intermittent asthma, and 25 atopic children with stable mild-to-moderate persistent asthma who were receiving inhaled corticosteroids (Table I). Atopic nonasthmatic children and atopic asthmatic children were recruited from the Allergy outpatient Clinic of the Istituto Dermopatico dell'Immacolata, IDI, Rome, Italy, and the Asthma and Allergy clinic of the Pediatric Hospital Bambino Gesù, Palidoro, Italy. The diagnosis and classification of asthma was based on clinical history and examination and pulmonary function parameters according to the "Guidelines for the diagnosis and management of asthma" issued by the National Heart, Lung, and Blood Institute of the National Institutes of Health.¹³ Briefly, steroid-naïve atopic asthmatic children had mild intermittent asthma with symptoms less often than twice a week, FEV₁ of 80% or greater of predicted value and reversibility of 12% or greater to salbutamol, or a positive provocation test result with methacholine or exercise. They were not taking any regular medication but used inhaled short-acting β₂-agonists as needed for symptom relief. They were excluded from the study if they had used inhaled corticosteroids or LT receptor antagonists in the previous 4

weeks. Steroid-treated atopic asthmatic children had persistent mild-to-moderate asthma and were receiving maintenance therapy with low-to-medium doses of inhaled corticosteroids (fluticasone propionate administered by means of spacer or diskus [GSK, Uxbridge, United Kingdom] and budesonide administered by means of Turbohaler [AstraZeneca, London, United Kingdom]) at a constant dose for at least 2 months. The overall mean daily dose of inhaled corticosteroids was 300 μg/day. They had not been taking LT receptor antagonists for at least 1 month.

Atopic nonasthmatic children had a clinical history of atopy and positive skin test results. All had allergic rhinitis and were either sensitized to perennial allergens or were recently exposed to a relevant allergen.

Healthy control children without any history of asthma and atopic disease were recruited from children of staff. They had negative skin prick test results and normal spirometry results.

Study group children had no upper respiratory tract infections in the previous 3 weeks. Children were excluded from the study if they had used oral steroids in the last 4 weeks or nonsteroidal anti-inflammatory drugs in the last 2 weeks.

Study design

Two studies were performed. The first study was of cross-sectional design. Children attended the Asthma outpatient clinic and the Department of Pharmacology of the Catholic University of the Sacred Heart, Rome, Italy, on one occasion for clinical examination, measurement of exhaled NO, collection of EBC, and spirometry.

A pilot uncontrolled open-label study was undertaken in steroid-naïve atopic asthmatic children to investigate the effect of inhaled corticosteroids on exhaled LTs and prostanoids. Fourteen children who were selected from the steroid-naïve atopic asthmatic group attended the Asthma outpatient clinic of the University Hospital A. Gemelli and the Department of Pharmacology of the Catholic University of the Sacred Heart, Rome, Italy, on 3 occasions for clinical examination, measurement of exhaled NO, and collection of EBC. After a screening visit (visit 1) and a 2-week run-in period (visit 2), children were given inhaled fluticasone propionate (100 μg) as dry powder (Flixotide Diskus) twice daily for 4 weeks. After treatment with fluticasone, children returned to the laboratory, and all the above tests were repeated (visit 3).

Repeatability for eicosanoid measurements was assessed in 14 children with asthma in a randomized design in which a second EBC sample was collected within 7 days of obtaining the first sample. Informed consent was obtained from parents, and the study was approved by the Ethics Committee of the Catholic University of the Sacred Heart, Rome, Italy.

Pulmonary function

FEV₁, forced vital capacity, and forced expiratory flow between 25% and 75% (FEF_{25%-75%}) were measured with a 10-L bell spirometer (Biomedin, Padova, Italy), and the best of 3 maneuvers, expressed as a percentage of predicted values, was chosen.

EBC collection

EBC was collected by using a condensing chamber (Ecoscreen; Jaeger, Hoechberg, Germany), as described previously.¹⁴ Briefly, exhaled air entered and left the chamber through 1-way valves at the inlet and outlet, thus keeping the chamber closed. Children were instructed to breathe tidally through a mouthpiece connected to the condenser for 15 minutes while wearing a nose clip. An average of 1.2 mL of EBC per child was collected and stored at -70°C before eicosanoid measurements. α -Amylase concentrations in all EBC samples were measured by means of an *in vitro* colorimetric method to check for possible salivary contamination (Roche Diagnostics, Basel, Switzerland).

Measurement of exhaled LTs and prostanoids

LTE₄ and LTB₄ concentrations in EBC were measured with specific enzyme immunoassay kits (Cayman Chemical, Ann Arbor, Mich).¹⁴ The detection limits were 4 pg/mL for LTB₄ and 8 pg/mL for LTE₄. The enzyme immunoassay for LTB₄ was previously validated by means of reverse-phase HPLC (RP-HPLC).¹⁵ LTE₄ antiserum cross-reactivity was 100% with LTE₅, 20% with N-acetyl-LTE₄, 10% with LTC₄, 9% with LTD₄, 5% with LTC₅, and 4% with LTD₅. The intra-assay and interassay coefficients of variation for the LTB₄ and LTE₄ assays were within 10% and 15%, respectively.

8-Isoprostane, PGE₂, and TxB₂ levels in EBC were measured with specific RIAs developed in our laboratory.^{16,17} RIA specificity for 8-isoprostane and PGE₂ has been recently confirmed by means of RP-HPLC.¹⁶ The detection limit for 8-isoprostane, PGE₂, and TxB₂ was 10 pg/mL. The intra-assay and interassay coefficients of variation for prostanoid RIAs were as follows: 8-isoprostane, less than 4% and less than 11%, respectively; PGE₂, less than 4% and less than 5%, respectively; and TxB₂, less than 5% and less than 10%, respectively.

LT and prostanoid values in EBC were expressed as the total amount (in picograms) of eicosanoids expired in the 15-minute breath test (eicosanoid concentrations \times volume of EBC).

Exhaled NO measurement

Exhaled NO levels were measured with the NIOX system (Aerocrine, Stockholm, Sweden) by using a single-breath online method, according to American Thoracic Society guidelines.¹¹ Children inhaled NO-free air and exhaled through a dynamic flow restrictor. Children were asked to exhale to residual volume, insert the mouthpiece, inhale to total lung capacity through an NO filter, and then exhale into the device through the same mouthpiece for 10 seconds at a constant flow of 50 mL/sec with the aid of visual feedback on a computer screen. A negative pressure tracing on the screen was used to confirm that children were inhaling NO-free air. Exhalations were repeated after a 1-minute relaxation period until the performance of 3 exhaled NO values varied less than 10%. Exhaled NO measurements were obtained before spirometry.

Skin testing

Atopy was assessed by using skin prick tests for common aeroallergens (mixture for house dust mite [*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*], grass pollen [cockfoot and timothy], tree pollen [birch, ash tree, olive tree, oak, and cypress], weed pollen [*Ambrosia artemisiifolia* and *Parietaria*

officinalis], animal danders [cat and dog allergens], and fungal allergens [*Aspergillus* species and *Alternaria alternata*]; Stallergenes, Antony, France). A positive skin test response was defined as a wheal with a mean diameter (mean of maximum and 90° midpoint diameters) of at least 3 mm greater than that produced with a saline control.

Statistical analysis

Eicosanoid concentrations in EBC and exhaled NO values were expressed as medians and interquartile ranges (25th–75th percentiles). Kruskal-Wallis tests followed by pairwise Mann-Whitney *U* tests were used to compare groups. Correlation was expressed as the Spearman coefficient. Significance was defined at a *P* value of less than .05.

In the fluticasone trial eicosanoid concentrations in EBC within the group and comparisons before and after treatment were analyzed by using the Friedman repeated-measures ANOVA. The study sample was calculated¹⁸ by considering the LTE₄ concentrations in EBC as the primary outcome. Sample size was estimated to be 14 children on the basis of exhaled LTE₄ concentrations after having considered an SD of 9.2 pg/15 min and a dropout rate of 20% and after having identified the minimal difference of biologic significance (12 pg/15 min) with a power of 90% (α value of 5% and β value of 10%).

Within-subject repeatability of exhaled eicosanoid measurements was expressed as the limits of agreement (mean difference \pm 2 SDs of the differences), according to the method of Bland and Altman.¹⁹ Precision of estimated limits of agreement was expressed as the SE of the limits of agreement.

RESULTS

No α -amylase concentrations were detected in any study sample, excluding significant salivary contamination.

Repeatability of measurements

Limits of agreement were as follows: LTE₄, 12.4 pg and -15.6 pg; LTB₄, 9.8 pg and -8.4 pg; 8-isoprostane, 5.6 pg and -5.6 pg; PGE₂, 4.9 pg and -4.8 pg; TxB₂, 4.7 pg and -3.7 pg (see Figs E1 and E2 in the Journal's Online Repository at www.mosby.com/jaci). The precision of limits of agreement was 3.3 pg for LTE₄, 2.2 pg for LTB₄, 1.9 pg for 8-isoprostane, 1.1 pg for PGE₂, and 1.1 pg for TxB₂.

Cross-sectional study

LTE₄, LTB₄, and 8-isoprostane concentrations were detectable in all healthy children and were increased in both steroid-naïve (LTE₄, $P < .001$; LTB₄, $P < .001$; and 8-isoprostane, $P < .001$) and steroid-treated atopic asthmatic children (LTE₄, $P < .001$; LTB₄, $P < .001$; and 8-isoprostane, $P < .001$) but not in atopic nonasthmatic children (LTE₄, $P = .14$; LTB₄, $P = .23$; and 8-isoprostane, $P = .52$; Fig 1; Fig 2, A; and Table II). Asthmatic children had higher concentrations of exhaled LTE₄ (steroid-naïve children, $P < .001$; steroid-treated children, $P < .001$), LTB₄ (steroid-naïve children, $P < .001$; steroid-treated children, $P < .001$), and 8-isoprostane (steroid-naïve children, $P < .001$; steroid-treated

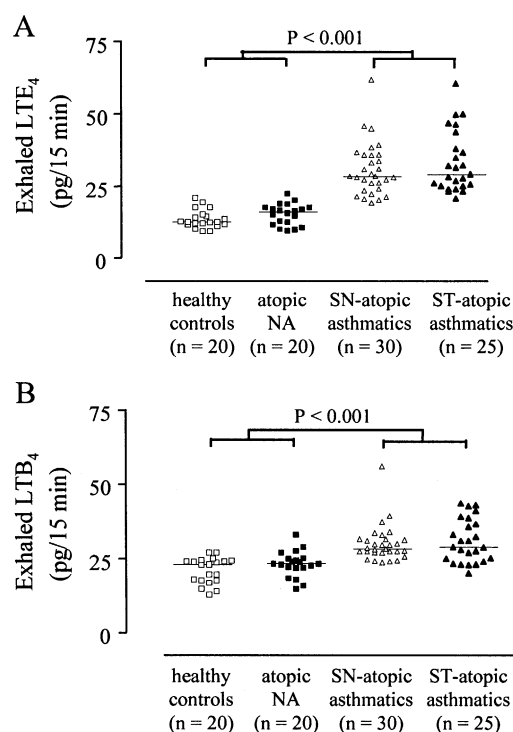


FIG 1. LTE_4 (A) and LTB_4 (B) concentrations in EBC in healthy children (open squares), atopic nonasthmatic (NA) children (filled squares), steroid-naïve (SN) atopic asthmatic children (open triangles), and steroid-treated (ST) children with atopic asthma (filled triangles). LT values are expressed as picograms produced during 15 minutes of breathing. Median values are shown with horizontal bars.

children, $P < .001$) than atopic nonasthmatic children (Fig 1; Fig 2, A; and Table II). There was no difference in exhaled LTE_4 , LTB_4 , and 8-isoprostane concentrations between the 2 groups of asthmatic children (LTE_4 , $P = .52$; LTB_4 , $P = .78$; and 8-isoprostane, $P = .36$; Fig 1; Fig 2, A; and Table II). PGE_2 concentrations in EBC were detectable in all children and were similar in the 4 study groups ($P = .61$; Fig 2, B, and Table II). TxB_2 was measurable in 8 healthy children, 7 atopic nonasthmatic children, 24 steroid-naïve atopic asthmatic children, and 20 steroid-treated atopic children with asthma (Fig 2, C). In those children in whom TxB_2 was measurable, there was no difference in its levels between the study groups (Fig 2, C, and Table II).

Compared with healthy children, mean exhaled NO levels were increased in atopic nonasthmatic children ($P < .01$) and, to a greater extent, in steroid-naïve atopic asthmatic children ($P < .001$) but not in atopic asthmatic children who were receiving inhaled corticosteroids ($P = .14$, Fig 3). However, 5 steroid-treated asthmatic children had exhaled NO levels of greater than 20 ppb. Exhaled NO levels were higher in steroid-naïve atopic asthmatic children than in atopic nonasthmatic children ($P < .01$, Fig 3). There was no correlation between exhaled eicosanoids or exhaled NO levels and age, sex, and lung

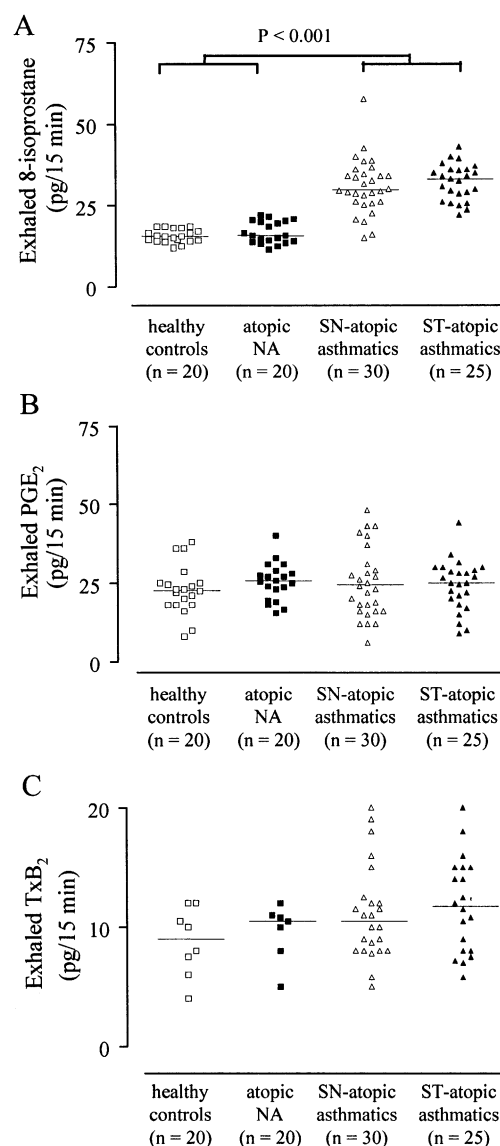


FIG 2. 8-Isoprostane (A), PGE_2 (B), and TxB_2 (C) concentrations in EBC in healthy children (open squares), atopic nonasthmatic (NA) children (filled squares), steroid-naïve (SN) atopic asthmatic children (open triangles), and steroid-treated (ST) children with atopic asthma (filled triangles). Prostanoid values are expressed as picograms produced during 15 minutes of breathing. Median values are shown with horizontal bars.

function in any study group. There was no correlation between the different exhaled markers in any study groups. There was no correlation between exhaled eicosanoid concentrations or exhaled NO levels and inhaled corticosteroid dose in those children who were treated with these drugs.

Interventional study

One child was excluded from the study because of asthma exacerbation during treatment with inhaled fluticasone. Values of exhaled LTs, prostanoids, and NO at

TABLE II. Exhaled eicosanoids and exhaled NO in study group children

	Healthy Children	Atopic nonasthmatic children	Atopic steroid-naive asthmatic children	Atopic steroid-treated asthmatic children
Exhaled eicosanoids (pg/15 min)				
LTE ₄	12.5 (11.6-14.7)	15.9 (12.0-17.5)	28.2 (24.9-35.7)†	29.0 (25.0-36.6)†
LTB ₄	23.0 (17.7-24.2)	23.3 (21.9-25.1)	28.4 (27.3-31.4)†	29.0 (25.0-35.7)†
8-Isoprostane	15.5 (14.1-17.5)	15.8 (13.9-20.1)	29.8 (26.0-34.3)†	33.0 (28.5-35.8)†
PGE ₂	22.8 (18.0-25.0)	25.7 (21.3-28.5)	24.5 (17.0-34.0)	25.0 (20.0-28.5)
TxB ₂	9.0 (6.8-11.3)	9.5 (9.0-10.9)	10.5 (8.0-12.3)	11.8 (8.0-15.0)
Exhaled NO (ppb)	7.1 (5.5-9.2)	13.9 (12.5-15.1)*	31.0 (26.3-42.5)†	9.5 (6.8-12.9)

Values are medians and interquartile ranges (25th-75th percentiles).
* $P < .01$ and † $P < .001$ compared with healthy children.

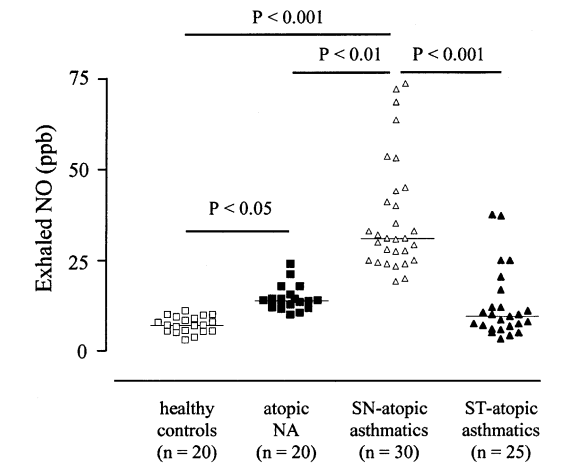


FIG 3. Exhaled NO levels in healthy children (open squares), atopic nonasthmatic (NA) children (filled squares), steroid-naive (SN) atopic asthmatic children (open triangles), and steroid-treated (ST) children with atopic asthma (filled triangles). Median values are shown with horizontal bars.

baseline and after a 2-week run-in period were similar (LTE₄, 36.6 pg [33.2-41.4 pg] vs 35.5 pg [33.4-36.2 pg], $P = .77$, respectively [Fig 4, A]; LTB₄, 28.8 pg [27.0-34.0 pg] vs 29.9 pg [28.2-31.1 pg], $P = .63$; 8-isoprostane, 29.4 pg [25.3-32.0 pg] vs 31.5 pg [27.0-35.5 pg], $P = .63$; PGE₂, 62.5 pg [59.0-65.3 pg] vs 61.0 pg [58.5-66.0 pg], $P = .69$; TxB₂, 8.3 pg [7.8-9.8 pg] vs 10.0 pg [7.5-10.8 pg], $P = .49$; NO, 31.0 ppb [26.0-41.0 ppb] vs 33.5 ppb [24.3-39.7 ppb], $P = .15$ [Fig 4, B]). LTE₄ concentrations in EBC were reduced by 18% after fluticasone treatment (before treatment, 35.5 pg [33.4-36.2 pg]; after treatment, 29.2 pg [27.5-33.2 pg]; $P < .01$; Fig 4, A), whereas exhaled NO levels were reduced by 53% (before treatment, 33.5 ppb [24.3-39.7 ppb]; after treatment, 15.8 ppb [12.3-20.9 ppb]; $P < .0001$; Fig 4, B). Fluticasone had no effect on exhaled LTB₄ ($P = .43$), 8-isoprostane ($P = .28$), PGE₂ ($P = .32$), and TxB₂ ($P = .56$) concentrations.

DISCUSSION

Because its capture is completely noninvasive, EBC is potentially useful for long-term monitoring of lung in-

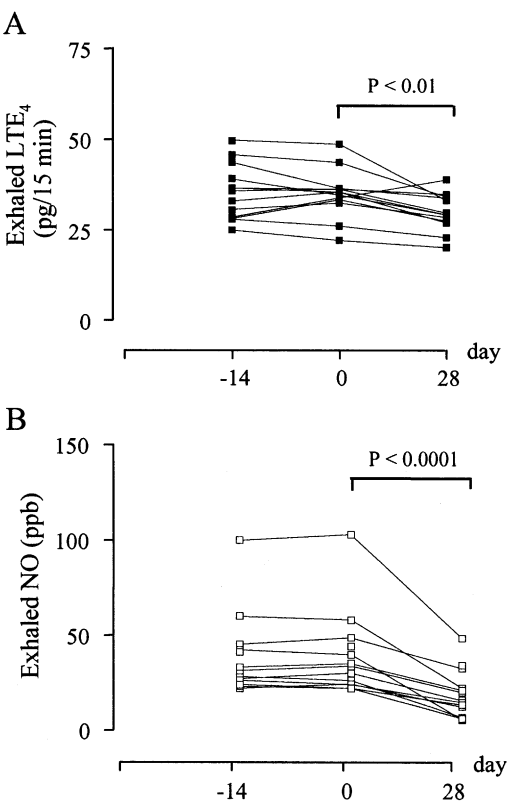


FIG 4. LTE₄ concentrations in EBC (A) and exhaled NO levels (B) in 13 steroid-naive atopic children with asthma at baseline (day -14), after 2-week run-in period (day 0), and after treatment with inhaled fluticasone (100 µg twice a day for 4 weeks). Values are expressed as medians.

flammation in children with asthma,²⁰ but there is no information on the utility of this technique in atopic nonasthmatic children or whether atopy per se affects the measurements of exhaled eicosanoids. Previous studies reported increased concentrations of LTs and 8-isoprostane in EBC in children with asthma, but these values were not compared with those in atopic nonasthmatic children.^{6-8,10} Exhaled NO is a well-accepted and well-validated marker of inflammation that correlates with peripheral blood eosinophils and bronchial hyperresponsiveness in children.²¹ Some studies reported increased

exhaled NO levels in atopic nonasthmatic children, particularly in those with rhinitis,^{22,23} but this issue is still open.

We have characterized the profile of LTs and prostanoids in EBC in atopic nonasthmatic children and in atopic children with stable asthma who were steroid naive or treated with inhaled corticosteroids. Atopic asthmatic children had normal FEV₁ values but decreased FEV₁/forced vital capacity ratios and FEF_{25%-75%} values, which are more sensitive for detecting reduced lung function in children. Compared with healthy control children, LTE₄, LTB₄, and 8-isoprostane concentrations were selectively increased in children with atopic asthma but not in atopic nonasthmatic children. By contrast, exhaled NO levels were increased in atopic nonasthmatic children and, to a greater extent, in steroid-naive atopic asthmatic children. These data suggest a different biologic significance for LTs and 8-isoprostane in EBC and exhaled NO because the former might be considered as markers of asthma, whereas the latter might reflect the different degree of inflammation within the respiratory tract in atopic children with and without asthma. This might have important implications for the diagnosis and treatment of children with allergic disease. Measurement of exhaled LTs might help identify those children with no current respiratory symptoms, well-maintained lung function, and increased exhaled NO levels who are not only atopic but who also have ongoing asthmatic inflammation that might require starting pharmacologic therapy. Moreover, increased exhaled NO levels in atopic nonasthmatic children with rhinitis reflect the presence of inflammation within the airways because the restricted exhalation method ensures velum closure, excluding contamination of the exhaled air with nasal NO.¹¹ This is consistent with a common origin for allergic rhinitis and asthma.¹ However, larger studies are needed to assess the biologic significance of these findings and their implications.

Similar exhaled LTE₄, LTB₄, and 8-isoprostane concentrations in the 2 asthmatic groups are consistent with the results of previous cross-sectional studies.⁶⁻⁸ Exhaled CysLT concentrations in asthmatic children were comparable with those previously reported,^{6,7} although in one study exhaled CysLTs were undetectable in asthmatic adults.²⁴ Comparisons of data obtained in different laboratories are currently difficult because of the lack of a standardized procedure for EBC collection and validated analytic techniques. This could be particularly relevant for chemically unstable compounds, such as CysLTs. EBC analysis is currently more reliable for relative measures than for determining absolute levels of inflammatory mediators.

There are no interventional studies on the effect of inhaled corticosteroids on exhaled LTs and prostanoids in children with stable asthma. In a previous uncontrolled open-label study, exhaled CysLT concentrations in children with asthma exacerbation were reduced after oral prednisone.⁹ In the present study, after treatment with inhaled fluticasone, LTE₄ concentrations in EBC were reduced by 18%, whereas concentrations of other exhaled eicosanoids

were unchanged. These results provide evidence that inhaled corticosteroid treatment at a daily dose of 200 µg has a limited effect on exhaled LTE₄ concentrations and no effect on other exhaled eicosanoid concentrations in children with stable asthma. The previously reported effect of corticosteroids on exhaled CysLTs in children with asthma exacerbations⁹ could be partly explained by the different route of administration (oral vs inhaled) and the different severity of inflammation (acute exacerbation vs stable asthma). In contrast to exhaled LTE₄ concentrations, exhaled NO levels were decreased by 53% after inhaled fluticasone, which is consistent with the fact that exhaled NO is markedly sensitive to corticosteroids.²⁵ Taken together, these data indicate that exhaled NO and LT concentrations in EBC might reflect different aspects of airway inflammation in asthmatic children. Increased exhaled LTE₄, LTB₄, and 8-isoprostane concentrations might indicate persisting airway inflammation in asthmatic children treated with inhaled corticosteroids. This might explain the additive effect of montelukast in asthmatic children maintained on inhaled corticosteroids.²⁶ However, in another study montelukast failed to decrease exhaled NO in asthmatic children treated with inhaled budesonide.²⁷ Our findings indicate that inhaled fluticasone has a limited effect on exhaled LTE₄ concentrations and no effect on exhaled LTB₄ and 8-isoprostane concentrations. This is consistent with preliminary investigations indicating that corticosteroids might partially block the synthesis of CysLTs,²⁸ possibly through a selective effect on LTC₄ synthase but not on LTA₄ hydrolase, which synthesizes LTB₄.²⁹ However, the possibility of an inadequate deposition of the inhaled fluticasone powder into the smaller airways of asthmatic children and a possible effect of higher doses should also be considered when interpreting these results. Moreover, the open-label uncontrolled study design limits the strength of our trial and precludes definitive conclusions. Further larger and controlled studies are needed to definitively establish the effects of inhaled corticosteroids on exhaled LTs and prostanoids in children with stable asthma.

Another current limitation of the EBC analysis is the lack of validation of the immunoassays for exhaled markers with more specific analytic techniques. We have recently confirmed that exhaled LTD₄, LTE₄, and LTB₄ concentrations are increased in both steroid-naive asthmatic adults and children by using gas chromatography-mass spectrometry, which is the reference analytic technique.¹⁰ Moreover, the specificity of the enzyme immunoassay for LTB₄ and the RIAs for 8-isoprostane and PGE₂, which were used in the present study, was previously demonstrated by RP-HPLC.^{15,16} Day-to-day repeatability of exhaled eicosanoid measurements is acceptable, as indicated by the limits of agreement for the different compounds and similar values for exhaled eicosanoids before and after the run-in period in the trial with inhaled fluticasone. However, there are several possible sources of variability in exhaled LT measurement, including differences in EBC collection, sample pretreatment and storage, analytic techniques, and severity

of asthma at the time of EBC collection.² To adjust for possible variations in the EBC sample volume, LT and prostanoid values in EBC were expressed as the total amount of the eicosanoid expired over a standard period of collection. There are no studies on the flow dependency of exhaled LTs. In a previous study in asthmatic adults, exhaled LT concentrations were not dependent on respiratory patterns.¹⁴ However, studies aiming at measuring exhaled LT concentrations at different flow rates under controlled conditions are required. Another limitation of the present study is that a reference dilution indicator was not used. As pointed out by Effros et al,³⁰ part of the variation in nonvolatile compound concentrations in EBC might be related to differences in the dilution of respiratory droplets by water vapor. The lack of correlation between structurally related compounds, such as LTB₄ and LTE₄, in the present study and the selective increase of prostanoids (8-isoprostane vs PGE₂) in the single subject previously reported³¹ do not seem to support this evidence. However, reference indicators, such as measurement of conductivity, as proposed by Effros et al,³² should be used in future studies aiming at quantifying exhaled eicosanoids.

In conclusion, we have shown that exhaled LT and 8-isoprostane concentrations are increased in atopic asthmatic children but not in atopic nonasthmatic children. Exhaled NO levels are increased in atopic nonasthmatic children and, to a greater extent, in atopic asthmatic children. Exhaled NO levels in asthmatic children are markedly reduced by inhaled corticosteroid treatment, whereas exhaled LTE₄ concentrations are slightly reduced and exhaled LTB₄ and 8-isoprostane concentrations are not reduced. This evidence supports a different biologic significance for exhaled NO and exhaled eicosanoids as markers of airway inflammation, which might have important diagnostic and therapeutic implications for children with atopy or atopic asthma.

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