

Maternal smoking affects lung function and airway inflammation in young children with multiple-trigger wheeze

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Background: Exposure to tobacco smoke is a well-known risk factor for childhood asthma and reduced lung function, but the effect on airway inflammation in preschool-aged children is unclear.

Objective: To examine the effect of parental smoking on lung function and fractional concentration of exhaled nitric oxide (FENO) in relation to both parental reports and children's urine cotinine concentrations in preschool-aged children with multiple-trigger wheeze.

Methods: A total of 105 3- to 7-year-old children with multiple-trigger wheeze and lung function abnormalities were recruited. Lung function was assessed by impulse oscillometry, and FENO measurements were performed. Exposure to tobacco smoke was determined by parental reports and measurement of children's urinary cotinine concentrations.

Results: Forty-three percent of the children were exposed to environmental tobacco smoke according to parental reports. The FENO level was significantly higher in children with a smoking mother ($n = 27$) than in children with a nonsmoking mother (23.4 vs 12.5 ppb, $P = .006$). The FENO level expressed as z score and the cotinine level correlated significantly ($P = .03$). Respiratory resistance at 5 Hz was higher in children exposed to maternal smoking than in others (0.99 vs 0.88 kPas/L, $P = .005$). Urinary cotinine concentrations reflected well parental reports on their daily smoking and increased relative to the number of cigarettes smoked in the family ($P < .01$). Atopy was found in 75% of the children, but it was not associated with the FENO value ($P = .65$).

Conclusion: Maternal smoking was associated with increased FENO value and poorer lung function in steroid-naïve preschool children with multiple-trigger wheeze. Larger controlled trials are needed to generalize the results. (J Allergy Clin Immunol 2013;■■■:■■■-■■■.)

Key words: Pediatric asthma, environmental tobacco smoke, exhaled nitric oxide, cotinine, lung function

Asthma is a chronic inflammatory airway disease involving complex interactions of cells and mediators. Modulation of inflammatory processes occurs not only as a result of endogenous asthmatic events but also from inhalation of exogenous environmental oxidants¹ such as environmental tobacco smoke (ETS), which is a well-known etiological factor in childhood asthma.²⁻⁵

Parental smoking has been associated with increased asthma incidence and poorer lung function in asthmatic children.^{5,6} Exposure to tobacco smoke products during pregnancy has been shown to reduce lung function in infants.^{4,5,7,8} Asthmatic children experience more exacerbations as well as wheezing episodes, lower respiratory tract infections, and otitis media if they have been exposed to tobacco smoke.^{2,5,6}

The fractional concentration of exhaled nitric oxide (FENO) is considered to reflect the extent of asthmatic airway inflammation.⁹ Some airway irritants such as ETS can also interfere with the results of the FENO measurement.¹⁰ Active smoking and decreased FENO values have been detected in adults.^{11,12} Acute exposure to cigarette smoke also produces a decrease in FENO levels.^{11,12}

In healthy children, no association between ETS and FENO levels has been found.¹³⁻¹⁵ In asthmatic children, nevertheless, controversial results have been reported.^{6,16-18} One study in young children with respiratory symptoms found an association between parental smoking and increased FENO values,¹⁷ but a recent report showed a negative effect of ETS on FENO values in school-aged children.¹⁶ Two studies found no association between ETS and FENO levels in asthmatic children.^{13,15} The effect of ETS on airway inflammation in preschool-aged children is unclear.

The exposure to tobacco smoke in children is most often assessed by parental reports of their smoking habits. Even reliable parental reports can be inaccurate as a measure of children's actual inhalation of smoke. Instead, cotinine, a major nicotine metabolite, can be used as a measure of nicotine intake in second-hand smoking. Cotinine level determination in serum, saliva, urine, or hair has been used to monitor exposure to tobacco smoke.^{19,20}

The definition of early wheezing symptoms as episodic (viral) and multiple-trigger wheeze phenotypes is recommended.²¹ Episodic wheezers wheeze periodically and are well between the episodes, but multiple-trigger wheezers also wheeze between the episodes in response to other than viral infection triggers.^{21,22} Multiple-trigger wheezers suffer from symptoms caused by irritants such as ETS. There are, however, little data to support that such irritants could directly deteriorate lung function, and systematic studies for this association are warranted.²¹ We hypothesized that parental smoking reduces lung function and increases

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This work was supported by Helsinki University Central Hospital Research Funds, the Sigrid Juselius Foundation, and Nummela Sanatoriums Stiftelse.

Disclosure of potential conflict of interest: S. Kalliola has received research support from Helsinki University Central Hospital Research Funds, the Sigrid Juselius Foundation, and Nummela Sanatoriums Stiftelse. L.P. Malmberg has received lecture fees from OrionPharma and GlaxoSmithKline. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication November 27, 2012; revised January 11, 2013; accepted for publication January 11, 2013.

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0091-6749/\$36.00

© 2013 American Academy of Allergy, Asthma & Immunology
http://dx.doi.org/10.1016/j.jaci.2013.01.005

Abbreviations used

ETS: Environmental tobacco smoke
 FENO: Fractional concentration of exhaled nitric oxide
 IOS: Impulse oscillometry
 Rrs5: Respiratory system resistance at 5 Hz

bronchial inflammation in preschool-aged children with multiple-trigger wheeze.

METHODS**Patients**

Children referred to the Division of Allergy, Helsinki University Central Hospital because of suspected asthma were recruited ($n = 105$). Children participated in the trial evaluating the efficacy of the combination of salmeterol with fluticasone propionate compared with fluticasone propionate or salmeterol alone in reducing bronchial inflammation in preschool children with multiple-trigger wheeze.²³ Study children were aged 3 to 7 years of age with multiple-trigger wheeze and had a history of wheeze or breathlessness both during and outside discrete episodes. The median duration of symptoms was 16 (2-58) months. All children had significant bronchodilator responsiveness and/or exercise-induced bronchoconstriction measured by impulse oscillometry (IOS).²⁴⁻²⁶ Patients were excluded if they displayed seasonal symptoms only, had received systemic or inhaled corticosteroids in the previous 6 months, or had suffered signs of respiratory tract infection within the past 2 weeks. All patients had lung function measured by IOS during the first visit at the study clinic. If the patient fulfilled the inclusion criteria, the patient was invited to the study clinic to complete the study investigations. The randomization to 3 different treatment groups was done after a run-in phase. The lung function was assessed again after an 8-week treatment phase. The trial profile is provided in the article published previously.²³ The study was approved by the ethics committee of Helsinki University Central Hospital, and written informed consent was obtained from the parents.

At enrollment, each parent filled in a questionnaire that defined background information and the number of cigarettes smoked in the family per day.

Lung function

Lung function tests were performed by using IOS, and respiratory system resistance at 5 Hz (Rrs5) and respiratory system reactance at 5 and 10 Hz were determined as described previously.²⁴⁻²⁶ Bronchodilator responsiveness was assessed after the inhalation of 300 μ g of salbutamol, a positive response being defined as a decrease of 35% or more in Rrs5.²⁴ Typical symptoms and an increase of more than 35% in Rrs5 following a free running test were considered as evidence of exercise-induced bronchoconstriction.²⁵

FENO level measurements

Measurements of FENO levels were performed with the stationary chemiluminescence-based analyzer NIOX (Aerocrine AB, Solna, Sweden) according to the American Thoracic Society recommendations.²⁷ The analyzer was calibrated according to the manufacturer's specifications. Children were seated, without a nose clip, and were asked to fill their lungs completely with NO-free air, and thereafter to exhale with a mean and instantaneous flow of 50 ± 5 mL/s for at least 6 seconds. Consecutive, acceptable paired measurements of FENO levels were recorded, and the repeatability and mean results were calculated. The device contains a dynamic flow control that maintains a constant rate during exhalation. The FENO values are reported as absolute values and z scores (SD from predicted).²⁸

Atopy

Children were screened by using skin prick tests for the following aeroallergens: birch, timothy grass, meadow fescue, mugwort, cladosporium

herbarum, cat, dog, horse, cow, house-dust mite, and the following foods: egg, milk, fish, wheat, shrimp, and peanut.

Skin prick test positivity was defined as a wheal with a diameter of 3 mm or more against at least 1 of the tested allergens.

Blood samples to assess eosinophil count and eosinophilic cationic protein were taken during the first or second visit at the study clinic and examined by the routine laboratory methods used in Helsinki University Central Hospital.²³

Urinary cotinine measurements

Urinary samples (3×10 mL) were gathered during the second visit at the study clinic.²³ Urinary samples were stored at -26°C until analyzed. Cotinine was extracted from urine by using the gas chromatographic method of Feyerabend and Russell.²⁰ A total of 200 μ L of 5-methylcotinine (100 μ g/L in dichloromethane) as internal standard was added and mixed with 200 μ g of urine. Six hundred microliters of sodium hydroxide solution (5 mol/L), 40 μ L of antifoam/phenol red mixture (20 mg phenol red/100 μ L 5% antifoam), and 100 μ L of 1,2 dichloroethane were added and the mixture was centrifuged. The concentrated extract (2 μ L) was injected into a Hewlett-Packard FFAP silica capillary column of the Agilent 7890A gas chromatograph equipped with Agilent bios bead nitrogen-phosphorus detector. Helium was used as a carrier gas, and air and hydrogen were used as detector gases. The detector temperature was 330°C , and the current was 30 pA. Oven temperature was programmed from 80°C to 120°C at $40^{\circ}\text{C}/\text{min}$, from 120°C to 220°C at $12^{\circ}\text{C}/\text{min}$, and 220°C for 10 minutes. The sensitivity of the assay was 0.7 μ g/L.²⁰

Statistical analysis

Normal distribution was tested by using Shapiro-Wilks test. Because FENO, cotinine, and Rrs5 were not normally distributed, nonparametric tests were used. Parental smoking was categorized in 2 different ways: binomial variable according to maternal smoking (yes/no) and 4 categories by different smoking habits at home (no smoking, only father, only mother, and both). Categorical variables were compared by using Kruskal-Wallis test, Jonckheere-Terpstra test, or Mann-Whitney U test. Bivariate correlations were made by using Spearman correlation coefficient. Chi-square test was used to compare 2 categorical data. Data were analyzed by using SPSS, version 19.0 (SPSS, Inc, Chicago, Ill).

RESULTS

The characteristics of the study population are presented in **Tables I and II**. Urine samples from 97 of the 105 children were available for cotinine analysis, and the FENO level was measured in 96 children. Therefore, we had complete data for FENO level, lung function, and urine cotinine levels from 96 children.

According to parental reports, 41 (43%) children were being exposed to ETS. Smoking was reported by 27 mothers and 28 fathers, and both parents smoked in 14 families.

Cotinine concentrations and parental reports

The median cotinine concentration was less than 0.01 μ g/L (range, 0-17.1 μ g/L). **Fig 1, A**, shows urine cotinine concentrations in relation to exposure to ETS as reported by parents in the questionnaires. Cotinine concentrations (median, range) in children with only mother smoking ($n = 13$; median, 4.1 μ g/L; range, 0-9.9 μ g/L) and with both parents smoking ($n = 14$; median, 4.6 μ g/L; range, 0-17.1 μ g/L) were significantly higher than in children with nonsmoking parents ($n = 55$; median, <0.01 μ g/L; range, 0-13.9 μ g/L; $P < .001$). Interestingly, exposure of children to ETS was at a lower level in those families where only the father smoked ($n = 14$; median, cotinine <0.01 μ g/L; range, 0-4.4 μ g/L).

TABLE I. Characteristics of study children

No. of subjects	96
Males, n (%)	65 (67)
Females, n (%)	32 (33)
Age (y), mean (range)	5.6 (3.9-7.9)
Height (cm), mean (range)	115 (99-133)
Duration of symptoms (mo), median (range)	15 (2-58)
ETS, n (%)	
No ETS	55 (57)
ETS	41 (43)
Only mother	13 (13)
Only father	14 (15)
Both	14 (15)
Cotinine (μg/L), median (range)	<0.01 (0-17.1)
Skin prick test result positive, n (%)	72 (75)
Rrs5 (kPas/L), median (range)	0.88 (0.54-2.05)
FENO level (ppb), median (range)	14.6 (3.5-120.7)
Parental atopy (%)	87
Mother	59
Father	65
Parental asthma (%)	34
Animals at home (%)	16

TABLE II. Characteristics of study children in 2 groups: ETS exposed and non-ETS exposed

	ETS (n = 41)	Non-ETS (n = 55)	P value
Males	27 (66)	38 (69)	.74
Age (y), mean (range)	5.6 (3.9-7.4)	5.6 (3.9-7.9)	.68
Duration of symptoms (mo), median (range)	13 (6-57)	16 (2-58)	.82
Height (cm), mean (range)	113.9 (98-129)	116.2 (99-133)	.1
Cotinine (μg/L), mean (range)	3.6 (0-17.1)	1.3 (0-13.9)	.001
Skin prick test positivity, n (%)	35 (85)	37 (67)	.043
Rrs5 (kPas/L), median (range)	0.99 (0.6-2.1)	0.84 (0.54-1.74)	.004
Rrs5, z score, median (range)	0.57 (−1.4 to 4.7)	−0.01 (−2.1 to 4.8)	.04
FENO level (ppb), median (range)	20 (5-120)	12.9 (3.5-93.9)	.084
FENO level, z score, median (range)	2.9 (0.1-6.7)	1.9 (−0.6 to 6.5)	.051
Parental atopy, n (%)	37 (90)	46 (85)	.46
Parental asthma, n (%)	16 (39)	16 (30)	.34
Animals at home, n (%)	12 (29)	3 (5.5)	.001

Taken altogether, cotinine levels increased in relation to the number of cigarettes smoked by either parent (Fig 1, B, $P < .01$).

FENO levels

The median FENO level was 14.6 (3.5-120.7) ppb, and the FENO value was significantly higher in children with a smoking mother ($n = 27$) than in children with a nonsmoking mother (23.4 vs 12.5 ppb, $P = .006$). The correlation between the FENO z score and the cotinine level was statistically significant ($P = .03$; Fig 2). Furthermore, the FENO level correlated positively with the number

of cigarettes smoked by the parents ($P = .05$), and the FENO level was higher in children with exposure to smoking only by the mother ($n = 13$; median, 23.3 ppb) or both parents ($n = 14$; median, 25.4 ppb) than in children with nonsmoking parents ($n = 55$; median, 12.9 ppb) or with only a father smoking ($n = 14$; median, 11.2 ppb; $P = .05$; Fig 1, C).

Lung function

The children underwent IOS for the assessment of lung function. Rrs5 was higher in children exposed to tobacco smoke via the mother than in children with nonsmoking mothers (0.99 vs 0.88 kPas/L, $P = .005$, Fig 1, D, 0.89 vs 0.31 z score, $P = .05$). The correlation between the cotinine level and Rrs5 did not reach the significant level. There was no significant correlation between cotinine levels or reported ETS and respiratory system reactance at 5 Hz or 10 Hz, bronchodilatation effect, or extent of the exercise-induced bronchoconstriction.

Atopy and ETS

Skin prick test result was positive in 75% of the children. Cotinine concentrations were not associated with eosinophilia, eosinophilic cationic protein, or skin prick test positivity. However, atopy was more pronounced in the children of smoking mothers than in children with a nonsmoking mother (93% vs 68%, $P = .01$). The eosinophil count was also higher in children with a smoking mother ($P = .04$), whereas atopy was not associated with FENO level in this population with high atopy prevalence ($P = .65$).

DISCUSSION

Two important and alarming messages emerge from this study. First, children with smoking mothers have clearly increased FENO levels. Second, despite their young age, these children have already developed decreased lung function. Our study also highlights the significance of dose; in other words, the urine cotinine concentrations correlated well with the parental reports of their daily smoking and FENO z score in this population with a high prevalence of parental smoking (43%).

Smoking is on the decline in Finland; according to national statistics, during the study years 2002 to 2005, 26% of men and 21% of women were smoking.²⁹ We found somewhat higher prevalence rates as 27% of mothers and 28% of fathers smoked in our sample of parents of children with multiple-trigger wheeze. The importance of ETS as a risk factor for childhood asthma is well established.^{1,30} ETS is a composition of gases and particles from burning cigarettes and exhaled mainstream smoke.³⁰ It contains about 4000 chemicals including carcinogens and more than 10^{14} oxidants per puff of smoke.¹ The particle size in ETS is smaller than in mainstream smoke, which means that it has a greater penetrability in the airways of children and thus may evoke airway symptoms. Smoking by the mother seems to be more harmful than smoking by the father. Similar findings have been reported earlier.⁵ Our results imply that preschool-aged children are often in proximity to their mothers even when mothers report smoking outside the house and less exposed to paternal smoking.

Most of the studies on ETS and children are based on parental reports of their smoking habits.^{16,17} We used a more exact

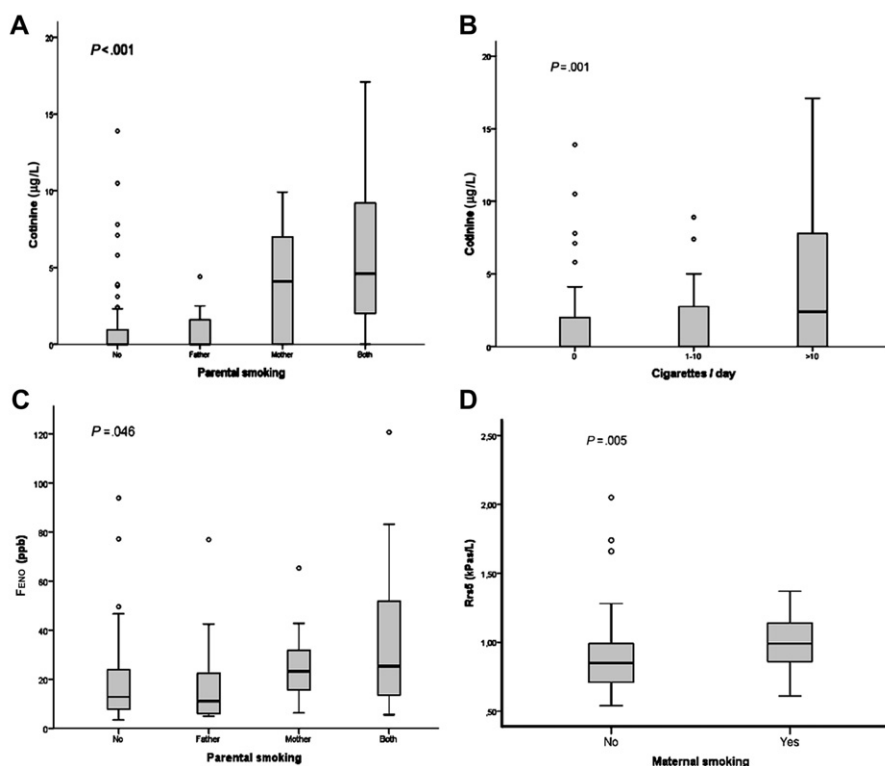


FIG 1. A, Urinary cotinine concentrations grouped according to the parental reports of their smoking habits. Cotinine concentrations were significantly higher in children with a smoking mother (median, 4.1 $\mu\text{g/L}$; range, 0–9.9 $\mu\text{g/L}$) or with both parents smoking (median, 4.6 $\mu\text{g/L}$; range, 0–17.1 $\mu\text{g/L}$) than in children with nonsmoking parents (median, <0.01 $\mu\text{g/L}$; range, 0–4.4 $\mu\text{g/L}$; $P < .001$ by Kruskal-Wallis test). *Open dots* indicate outliers. B, Median cotinine concentrations increased relative to the number of cigarettes smoked by either parent ($P < .001$, $R = 0.363$ by Kruskal-Wallis test). The outliers are represented by *open dots*. C, FENO levels in relation to parental reports of their daily smoking. FENO levels are medians (mother 23.3 ppb, both 25.4 ppb, nonsmoking 12.9 ppb, father 11.2 ppb, $P = .046$ by Jonckheere-Terpstra trend test). *Open dots* indicate the outliers. D, Rrs5 grouped according to the maternal smoking. The mean Rrs5 was higher in children exposed to tobacco smoke via the mother than in children with nonsmoking mothers (0.99 vs 0.88 kPa/L, $P = .005$ by Mann-Whitney test). *Open dots* indicate outliers.

monitoring of ETS by measuring urinary cotinine concentrations. Cotinine has a longer elimination half-time (about 20 h) compared with 2 hours for nicotine and therefore offers a better measure of nicotine intake during the previous few days.² As far as we are aware, there are only 2 previous studies that have compared cotinine concentrations with FENO levels in asthmatic children. Spanier et al⁶ studied 6- to 12-year-old children with doctor-diagnosed asthma. They found no association between cotinine levels or reported ETS and FENO levels. De la Riva-Velasco et al³¹ studied 8- to 18-year-old children with asthma on inhaled corticosteroids. ETS-exposed children had lower FENO levels than did non-ETS-exposed children. Both the studies had older patients than in our study, and the children were using inhaled corticosteroids. The strength of our study is that preschool children were steroid-naïve, preventing the possible masking effect by medication on the inflammatory response. Our study children were multiple-trigger wheezers whose symptoms are caused by many different triggers including exposure to tobacco smoke, which makes the parental smoking particularly harmful to these children. In these children, ETS can serve as one of the irritants that increase airway inflammation and FENO levels. Previous studies have not examined this effect in this age group.

Different analytical methods for cotinine concentrations have been postulated to monitor second-hand smoking. Immunoassays

such as radioimmunoassay and ELISA lack the sensitivity and specificity needed to detect low cotinine levels.³² The gas chromatographic method used here is highly sensitive and specific also for ETS monitoring (detection limit, 0.7 $\mu\text{g/L}$).³² The measured cotinine levels were relatively low as expected in cases of second-hand smoke exposure, and in 58 (60%) samples, there were undetectable cotinine concentrations. Still, the significant correlation between FENO levels expressed as z scores and the cotinine level could be assessed. The clinical implication of this finding is unclear. There was also a correlation between the cotinine concentrations in the children's urine and the number of cigarettes smoked by parents. This has also been shown in earlier studies.^{2,19}

Measurement of cotinine concentration in a single time spot can be affected by many different factors.^{19,33} Timing of the measurement of cotinine concentration and former exposure to tobacco smoke is important and can have an influence on the outcome. There may also be some race- and age-related differences in cotinine metabolism.³³ Although parental smoking is the principal source of ETS in children, they can be exposed to ETS in public places or by third-hand smoke. Third-hand smoke refers to the type of the ETS that appears after a cigarette has been extinguished and occurs via the inhalation of noxious gases and particles from smoked cigarettes or

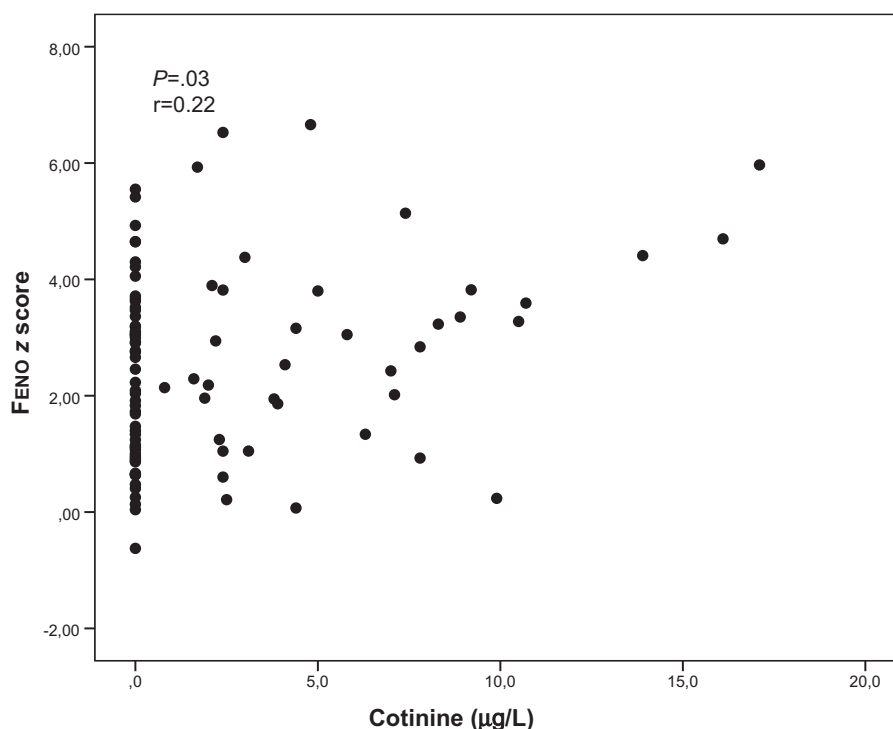


FIG 2. The children's urine cotinine concentration correlated with the FENO level expressed as z score ($P = .03$ by Spearman correlation test).

objects contaminated by absorbed tobacco smoke such as furniture or clothes.^{34,35}

We found an association between maternal smoking and increased FENO levels in these preschool-aged children with multiple-trigger wheeze. Previous studies in children have reported controversial results.^{16–18} In active adult smokers, FENO levels are lower than in nonsmokers.^{11,12} Tobacco smoke contains notable concentrations of NO, and acute ETS exposure is thought to cause a decrease in FENO levels by negative feedback on inducible NO synthase.¹⁰ Although acute exposure to tobacco smoke seems to evoke a reduction in FENO levels in adults, there are limited data of its long-term effects on airway inflammation, especially in small children. One study on young children found an association between an increased FENO value and parental-informed ETS.¹⁷ Laoudi et al¹⁶ observed reduced FENO levels in school-aged children with nontreated allergic asthma and who had a smoking parent. Perzanowski et al¹⁸ found an association between ETS exposure at age 4 years and increased FENO levels at age 7 years but an inverse association with present ETS exposure. The discrepancies in study results are most probably due to the small sample sizes, the heterogeneity of the study populations, and some specific age-related differences in inflammatory responses on the airway epithelium. A recent study showed strong T_H2 -type cytokine responses in infants with smoking parents.³⁶ Genetic differences may also explain part of the inconsistencies seen in the association between FENO levels and ETS.^{37,38}

Our study children were well-characterized multiple-trigger wheezers with lung function assessed by noninvasive IOS. While atopy has been claimed to be associated with FENO levels,^{9,16,26,28} in our study population, no such connection could be detected. This could be explained by this highly homogeneous study sample with steroid-naïve multiple-trigger wheezers and the FENO

levels were rather high in most of the study patients. Lung function was poorer in children with smoking mothers, which has also been shown by other investigators.^{3–5} It is possible that there is even a causal relationship between ETS and airway symptoms and reduced lung function.^{3–5} Confounding factors such as parental atopy and animals at home were examined and no connection to increased FENO levels or decreased lung function was found. Still, there can be some unmeasured socioeconomic factors that impacted the study results.

In the present study, parental smoking, particularly maternal smoking, was associated with increased FENO values in steroid-naïve preschool-aged children with multiple-trigger wheeze. Urinary cotinine concentrations reflected well parental reports about their smoking habits. Children with smoking mothers also had poorer lung function, in agreement with the detrimental effects of ETS. Further studies are needed to clarify the proinflammatory mechanisms evoked by ETS exposure in small children, but the findings and message of this study are clear—young children should not be exposed to ETS.

The authors thank specialist nurse Tuija Rito for her skill and care with the children, the children themselves, and their parents.

Clinical implications: Environmental tobacco smoke modifies bronchial inflammation and lung function of young children with mothers who smoke.

REFERENCES

1. Comhair SA, Gaston BM, Ricci KS, Hammel J, Dweik RA, Teague WG, et al. Detrimental effects of environmental tobacco smoke in relation to asthma severity. *PLoS One* 2011;6:e18574.

2. Chilmonczyk BA, Salmun LM, Megathlin KN, Neveux LM, Palomaki GE, Knight GJ, et al. Association between exposure to environmental tobacco smoke and exacerbations of asthma in children. *N Engl J Med* 1993;328:1665-9.
3. Carlsen KH, Lodrup Carlsen KC. Parental smoking and childhood asthma: clinical implications. *Treat Respir Med* 2005;4:337-46.
4. Carlsen KH, Carlsen KC. Respiratory effects of tobacco smoking on infants and young children. *Paediatr Respir Rev* 2008;9:11-9; quiz 19-20.
5. Cook DG, Strachan DP, Carey IM. Health effects of passive smoking, 9: parental smoking and spirometric indices in children. *Thorax* 1998;53:884-93.
6. Spanier AJ, Hornung R, Lierl M, Lanphear BP. Environmental exposures and exhaled nitric oxide in children with asthma. *J Pediatr* 2006;149:220-6.
7. Lodrup Carlsen KC, Jaakkola JJ, Nafstad P, Carlsen KH. In utero exposure to cigarette smoking influences lung function at birth. *Eur Respir J* 1997;10:1774-9.
8. Stick SM, Burton PR, Gurrin L, Sly PD, LeSouef PN. Effects of maternal smoking during pregnancy and a family history of asthma on respiratory function in newborn infants. *Lancet* 1996;348:1060-4.
9. Pijnenburg MW, De Jongste JC. Exhaled nitric oxide in childhood asthma: a review. *Clin Exp Allergy* 2008;38:246-59.
10. La Grutta S, Ferrante G, Malizia V, Cibella F, Viegi G. Environmental effects on fractional exhaled nitric oxide in allergic children. *J Allergy (Cairo)* 2012;2012: 916-26.
11. McSharry CP, McKay IC, Chaudhuri R, Livingston E, Fraser I, Thomson NC. Short and long-term effects of cigarette smoking independently influence exhaled nitric oxide concentration in asthma. *J Allergy Clin Immunol* 2005;116:88-93.
12. Kharitonov SA, Robbins RA, Yates D, Keatings V, Barnes PJ. Acute and chronic effects of cigarette smoking on exhaled nitric oxide. *Am J Respir Crit Care Med* 1995;152:609-12.
13. Barreto M, Villa MP, Martella S, Ronchetti F, Darder MT, Falasca C, 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.
14. Franklin PJ, Taplin R, Stick SM. A community study of exhaled nitric oxide in healthy children. *Am J Respir Crit Care Med* 1999;159:69-73.
15. Dinakar C, Lapuente M, Barnes C, Garg U. Real-life environmental tobacco exposure does not affect exhaled nitric oxide levels in asthmatic children. *J Asthma* 2005;42:113-8.
16. Laoudi Y, Nikasinovic L, Sahraoui F, Grimfeld A, Momas I, Just J. Passive smoking is a major determinant of exhaled nitric oxide levels in allergic asthmatic children. *Allergy* 2010;65:491-7.
17. Franklin PJ, Turner S, Mutch R, Stick SM. Parental smoking increases exhaled nitric oxide in young children. *Eur Respir J* 2006;28:730-3.
18. Perzanowski MS, Divjan A, Mellins RB, Canfield SM, Rosa MJ, Chew GL, et al. Exhaled NO among inner-city children in New York City. *J Asthma* 2010;47: 1015-21.
19. Ino T, Ohtani T, Yoshimi I. Urinary biomarkers for secondhand smoke. *J Clin Lab Anal* 2011;25:354-8.
20. Feyerabend C, Russell MA. A rapid gas-liquid chromatographic method for the determination of cotinine and nicotine in biological fluids. *J Pharm Pharmacol* 1990; 42:450-2.
21. Brand PL, Baraldi E, Bisgaard H, Boner AL, Castro-Rodriguez JA, Custovic A, et al. Definition, assessment and treatment of wheezing disorders in preschool children: an evidence-based approach. *Eur Respir J* 2008;32:1096-110.
22. Savenije OE, Kerkhof M, Koppelman GH, Postma DS. Predicting who will have asthma at school age among preschool children. *J Allergy Clin Immunol* 2012; 130:325-31.
23. Mäkelä MJ, Malmberg LP, Csonka P, Klemola T, Kajosaari M, Pelkonen AS. Sal-meterol and fluticasone in young children with multiple-trigger wheeze. *Ann Allergy Asthma Immunol* 2012;109:65-70.
24. Malmberg LP, Pelkonen A, Poussa T, Pohjanpalo A, Haahtela T, Turpeinen M. Determinants of respiratory system input impedance and bronchodilator response in healthy Finnish preschool children. *Clin Physiol Funct Imaging* 2002;22: 64-71.
25. Malmberg LP, Makela MJ, Mattila PS, Hammaren-Malmi S, Pelkonen AS. Exercise-induced changes in respiratory impedance in young wheezy children and non-atopic controls. *Pediatr Pulmonol* 2008;43:538-44.
26. Malmberg LP, Pelkonen AS, Mattila PS, Hammaren-Malmi S, Makela MJ. Exhaled nitric oxide and exercise-induced bronchoconstriction in young wheezy children – interactions with atopy. *Pediatr Allergy Immunol* 2009;20:673-8.
27. American Thoracic Society. European Respiratory Society. 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.
28. Malmberg LP, Pelkonen AS, Haahtela T, Turpeinen M. Exhaled nitric oxide rather than lung function distinguishes preschool children with probable asthma. *Thorax* 2003;58:494-9.
29. Statistics Finland. Tobacco statistics 2009. Available at: http://www.stat.fi/til/tup/2009/tup_2009_12-22.fi.pdf. Accessed January 24, 2013.
30. Cheraghi M, Salvi S. Environmental tobacco smoke (ETS) and respiratory health in children. *Eur J Pediatr* 2009;168:897-905.
31. de la Riva-Velasco E, Krishnan S, Dozor AJ. Relationship between exhaled nitric oxide and exposure to low-level environmental tobacco smoke in children with asthma on inhaled corticosteroids. *J Asthma* 2012;49:673-8.
32. Song L, Davis W, Abrams S, Hemiup J, Kazim L, Cummings K, et al. Sensitive and rapid method for the determination of urinary cotinine in non-smokers: an application for studies assessing exposures to second hand smoking (SHS). 2005;545: 200-8.
33. Rabinovitch N, Silveira L, Gelfand EW, Strand M. The response of children with asthma to ambient particulate is modified by tobacco smoke exposure. *Am J Respir Crit Care Med* 2011;184:1350-7.
34. Matt GE, Quintana PJ, Zakarian JM, Fortmann AL, Chatfield DA, Hoh E, et al. When smokers move out and non-smokers move in: residential thirdhand smoke pollution and exposure. *Tob Control* 2011;20:e1.
35. Jung JW, Ju YS, Kang HR. Association between parental smoking behavior and children's respiratory morbidity: 5-year study in an urban city of South Korea. *Pediatr Pulmonol* 2012;47:338-45.
36. Linnamaa P, Nieminen K, Koulou L, Tuomasjukka S, Kallio H, Yang B, et al. Pro-inflammatory and Th2-type cytokine responses in PBMC in infants are associated with parental smoking. *Clin Exp Allergy* 2012;42:1472-8.
37. Spanier AJ, Kahn RS, Hornung RW, Wang N, Sun G, Lierl MB, et al. Environmental exposures, nitric oxide synthase genes, and exhaled nitric oxide in asthmatic children. *Pediatr Pulmonol* 2009;44:812-9.
38. Salam MT, Bastain TM, Rappaport EB, Islam T, Berhane K, Gauderman WJ, et al. Genetic variations in nitric oxide synthase and arginase influence exhaled nitric oxide levels in children. *Allergy* 2011;66:412-9.