

ENVIRONMENTAL DETERMINANTS

Passive Smoking Induces Leukotriene Production in Children: Influence of Asthma

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Background. Passive smoking is associated with poor asthma control in children, but the mechanism is unknown. Leukotrienes are involved in the asthma pathogenesis and their synthesis is increased in adult subjects who actively smoke. **Objective.** To evaluate whether passive smoking, as assessed by urinary cotinine levels, increases leukotriene production in children with or without asthma. **Methods.** This was a prospective, cross-sectional study in which children with stable intermittent asthma (without exacerbation) and healthy control children were studied through spirometry and urinary concentrations of cotinine and leukotriene E₄ (LTE₄). Both groups were balanced to include children with and without passive smoking. **Results.** Ninety children (49 with asthma and 41 controls, 54.4% females) aged 9 years (range, 5–13 years) were studied. Urinary LTE₄ concentrations were progressively higher as cotinine levels increased ($r_s = 0.23$, $p = .03$). LTE₄ also correlated with body mass index (BMI) ($r_s = 0.30$, $p = .004$), and multiple regression analysis revealed that BMI was even more influential than cotinine for determining LTE₄ levels. LTE₄ concentrations were unrelated with gender, age, or spirometry. In turn, cotinine inversely correlated with forced expiratory volume in one second (FEV₁) ($r_s = -0.22$, $p = .04$) and forced vital capacity (FVC) ($r_s = -0.25$, $p = .02$), but when analyzed by groups, these relationships were statistically significant only in children with asthma. **Conclusions.** Exposure to environmental tobacco smoke, as assessed by urinary cotinine levels, was associated with an increased urinary concentration of LTE₄, although BMI exerted more influence in determining its concentration. Urinary cotinine was associated with decreased lung function, mainly in children with asthma.

Keywords body mass index, cotinine, environmental tobacco smoke, secondhand smoke, side-stream smoke

INTRODUCTION

Involuntary exposure to tobacco smoke, also called passive smoking, is a public health concern, especially at pediatric ages. The World Health Organization estimates that about 40% of the children are regularly exposed to environmental tobacco smoke worldwide (1), and in 2008, the Global Youth Tobacco Surveillance revealed that 42.5% of non-smoker adolescents were exposed to tobacco smoke in their own homes during the previous week (2). The prevalence of passive smoking among the children with some respiratory disease such as asthma is somewhat lower, but remains important: in Mexico, it is approximately 14.4% (3).

It is well documented that in patients with asthma, passive exposure to tobacco smoke augments the severity

of the disease and increases the number of exacerbations (4, 5). The mechanisms leading to these deleterious effects remain poorly understood, but there are some clues pointing to that tobacco-induced cysteinyl leukotrienes production may be one of these mechanisms. Within this context, Fauler and Frolich reported that among smoking adults, there was a close correlation between urinary excretion of leukotriene E₄ (LTE₄) and the daily number of cigarettes smoked (6). Furthermore, in this same report, urinary LTE₄ in healthy non-smokers nearly doubled baseline values after smoking the first half of 12 cigarettes at hourly intervals. Saareks et al. reported that adult smokers had up to five-fold higher levels of urinary LTE₄ when compared with non-smokers, but when the smokers suspended tobacco consumption, these levels dropped by 70% three days later (7). Rabinovitch et al. studied 27 asthmatic children with or without passive smoking and observed that increased excretion of urinary LTE₄ was associated with greater salbutamol use 2 days later, and this effect was

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more noticeable in children with highest urinary cotinine levels (8). More recently, this same research group reported that an increase in the urinary concentration of LTE₄ predicted the risk of asthma exacerbation in children with passive exposure to environmental tobacco smoke (as assessed by questionnaire and/or urinary cotinine), but not in children without passive smoking (9).

Thus, the aim of the present study was to investigate whether passive smoking, as assessed by urinary cotinine concentration, correlates with urinary LTE₄ levels in school-aged children and, if so, whether this correlation is different between children with asthma and control children without asthma.

MATERIALS AND METHODS

This was a prospective, cross-sectional, and observational study. Two groups were recruited: children with asthma and healthy control children; both groups were balanced to include children with and without passive smoking (defined as co-habitation with at least one active smoker who regularly smokes in the same room where the child stays or in a nearby room). Children with asthma were patients being cared for at a pediatric pulmonology service and with an established diagnosis of intermittent asthma according to international criteria (10), which included the following: (1) diurnal symptoms of asthma less than once a week, (2) brief exacerbations and asymptomatic in-between, (3) nocturnal symptoms, not more than two in 1 month, and (4) peak expiratory flow (PEF) $\geq 80\%$ of the predicted value. Children were excluded if they experienced asthmatic exacerbation in the last 30 days or has received corticosteroids (either systemic or inhaled), long-acting beta-adrenergics, or anti-leukotrienes (in the past 30 days), non-steroidal anti-inflammatory drugs (in the past 24 h), or rapid-acting bronchodilators (in the past 12 h). The control group consisted of children attending a nearby elementary school and who were free of clinical data of asthma and without a parental history of asthma, allergic rhinitis, or atopic eczema. In both groups, children with other chronic pulmonary, renal, hepatic, cardiac, or immunological diseases or those with infectious processes, major traumatism, or surgical procedures in the previous 14 days or who were currently receiving immunotherapy were also excluded from the study. An informed consent letter was signed by one of the children's parents. The protocol was approved by the research and bioethics committees of the Hospital de Pediatría, Instituto Mexicano del Seguro Social (approval number 2003/718/037) and the Instituto Nacional de Enfermedades Respiratorias (approval number C15-05).

Parents were asked to collect a urine sample of their child in a sterile flask on the day of the study, preferentially the first void in the morning, and to maintain it in the refrigerator or on ice until it was delivered to the researcher. Then, the urine sample was immediately introduced into liquid nitrogen until its study. On the same day, clinical and spirometric evaluations were performed.

Spirometry

With the child in the standing position, a spirometry was performed following international recommendations (11) and using commercial equipment (MicroLab, Micro Medical Ltd, Rochester, UK) calibrated on a daily basis. The results were expressed as the percentage of predicted values according to the Knudson formula (12).

LTE₄ and Cotinine Measurement

Urine samples were filtered through 0.22 μm membranes (Millipore, Billerica, MA, USA), and the cotinine and LTE₄ concentrations were measured by means of commercial ELISA (catalog no. CT086D; Calbiotech, Spring Valley, CA, USA) and EIA (catalog no. 520411; Cayman, Ann Arbor, MI, USA) kits, respectively. Plates were read in a spectrophotometer (Multiskan MS; Labsystem, Helsinki, Finland) at 450 and 620 nm for cotinine and at 405 nm for LTE₄.

Data Analysis

Because many variables did not follow a normal distribution, a non-parametric approach was used. Differences between children with asthma and control children were evaluated by the Mann-Whitney *U* or chi-square test. Spearman correlation coefficient (r_s) was employed to evaluate the association between urinary concentrations of LTE₄ and cotinine, both adjusted for the creatinine concentration (for correcting the dilution of the sample) and log-transformed. This same analysis was used to evaluate associations between LTE₄ or cotinine with other interval variables. Difference between two correlation coefficients was assessed with the procedure suggested by Zar (13). Multiple linear regression analysis was also utilized to simultaneously assess the influence of selected variables on LTE₄ levels or lung function. Statistical significance was set at $p \leq .05$ bimariginally. Data statistics were performed with SPSS v10 statistical software (SPSS Inc., Chicago, ILL, USA).

RESULTS

Ninety children (49 with asthma and 41 controls) aged between 5 and 13 years (median, 9 years of age), 54.4% females, were studied. The general characteristics of both groups are illustrated in Table 1, where it can be observed that the only statistically significant anthropometric difference was that body mass index (BMI), expressed as percentile for age, was slightly higher among children with asthma than in controls (median, 84.6 vs. 64.7, respectively, $p = .04$). Urinary levels of LTE₄ (336 vs. 368 pg/mg creatinine, respectively, $p = .15$) and cotinine (1.98 vs. 1.23 ng/mg creatinine, respectively, $p = .14$) were not different between children with asthma and controls. Lung function, as assessed by forced expiratory volume in one second (FEV₁) and FEV₁/forced vital capacity (FVC) was slightly lower in patients with asthma than in healthy children.

TABLE 1.—General characteristics of the study population and urinary levels of LTE₄ and cotinine.

Characteristic	Children with asthma (n = 49)	Control children (n = 41)	p ^a
Gender (F:M)	27:22	22:19	0.89
Age (years)	9 (6–13)	9 (5–12)	0.42
Weight (kg)	33.3 (20.8–78)	32.4 (18–86.5)	0.16
Height (cm)	136 (110–161)	133 (110–164)	0.20
BMI (kg/m ²)	18.1 (11.9–31.6)	17.6 (14.1–33.8)	0.10
BMI (percentile for age)	84.6 (0.2–100)	64.7 (12.6–100)	0.04
LTE ₄ (pg/mg creatinine)	336 (27–1980)	368 (51–2415)	0.15
Cotinine (ng/mg creatinine)	1.98 (0.04–38.45)	1.23 (0.05–25.97)	0.14
FEV ₁ (% predicted)	98 (71–140)	107 (79–156)	0.003
FVC (% predicted)	100 (74–148)	109 (79–160)	0.08
FEV ₁ /FVC (% predicted)	94 (61–110)	98 (74–107)	0.01

Note: Data correspond to median (minimum–maximum), except for gender, in frequency. BMI, body mass index; LTE₄, Leukotriene E₄; FEV₁, forced expiratory volume at one second; and FVC, forced vital capacity.

^aChi-square or Mann–Whitney *U* test.

TABLE 2.—Association between urinary LTE₄ and urinary cotinine with other variables.

	All children		Children with asthma		Control children	
	r _s	p	r _s	p	r _s	p
LTE ₄ (pg/mg creatinine) vs.						
Cotinine (ng/mg creatinine)	0.23	0.03	0.20	0.16	0.31	0.05
Age (years)	–0.02	0.84	–0.18	0.20	0.15	0.35
Weight (kg)	0.17	0.10	0.10	0.51	0.26	0.10
Height (cm)	0.07	0.50	0.003	0.98	0.18	0.26
BMI (percentile for age)	0.30	0.004	0.40	0.005	0.25	0.12
FEV ₁ (% predicted)	0.18	0.11	–0.04	0.80	0.26	0.11
FVC (% predicted)	0.14	0.21	–0.04	0.80	0.26	0.11
FEV ₁ /FVC (% predicted)	–0.02	0.89	–0.04	0.78	–0.06	0.73
Cotinine (ng/mg creatinine) vs.						
Age (years)	–0.20	0.06	–0.18	0.21	–0.16	0.32
BMI (percentile for age)	0.15	0.17	0.11	0.47	0.19	0.25
FEV ₁ (% predicted)	–0.22	0.04	–0.32	0.03	0.06	0.71
FVC (% predicted)	–0.25	0.02	–0.33	0.03	–0.04	0.83
FEV ₁ /FVC (% predicted)	0.11	0.33	0.07	0.66	0.14	0.39

Note: Data correspond to Spearman correlation coefficient (r_s) and its statistical significance (p). Leukotriene E₄ (LTE₄) and cotinine are in log-transformed values. BMI, body mass index; FEV₁, forced expiratory volume at one second; and FVC, forced vital capacity.

Association Between LTE₄ and Cotinine

Once LTE₄ and cotinine values were log-transformed, correlation analysis showed that urinary concentration of LTE₄ tended to be higher as the urinary concentration of cotinine increased (r_s = 0.23, p = .03, Table 2). However, when children with asthma and control children were analyzed separately, the correlation between cotinine and LTE₄ reached statistical significance only in non-asthmatic control children (r_s = 0.31, p = .05, Table 2 and Figure 1B), but not among patients with asthma (r_s = 0.20, p = .16, Table 2 and Figure 1A). This difference between both groups was relatively small, inasmuch as the two correlation coefficients were not statistically different (p = .60), and the multiple linear regression showed that the interaction term between cotinine and asthma was not predictive of the LTE₄ levels (Table 3).

Association Between LTE₄ and Other Variables

Urinary levels of LTE₄ were not different among males and females (340.2 [range, 50.9–2414.8] vs. 348.1 [range,

27.5–1693.3] pg/mg creatinine, respectively, p = .74), nor were they associated with age, weight, height, or lung function (Table 2). LTE₄ levels had a direct correlation with BMI (r_s = 0.30, p = .004), but when separated by groups, this association only persisted in children with asthma (Table 2 and Figure 2). Taking into account the entire study population, multiple linear regression showed that BMI was even more influential than cotinine in predicting LTE₄ levels (standardized beta coefficients, 0.334 and 0.262, respectively, Table 3).

Association Between Cotinine and Other Variables

Cotinine levels in urine were similar between males and females (1.2 [range, 0.1–26.1] vs. 2.1 [range, 0.04–38.5] ng/mg creatinine, p = .58). As can be observed in Table 2, cotinine exerted a negative impact on lung function, inasmuch as higher cotinine concentrations were associated with lower values of FEV₁ (r_s = –0.22, p = .04) and FVC (r_s = –0.25, p = .02). This relationship reached statistical significance among children with asthma but

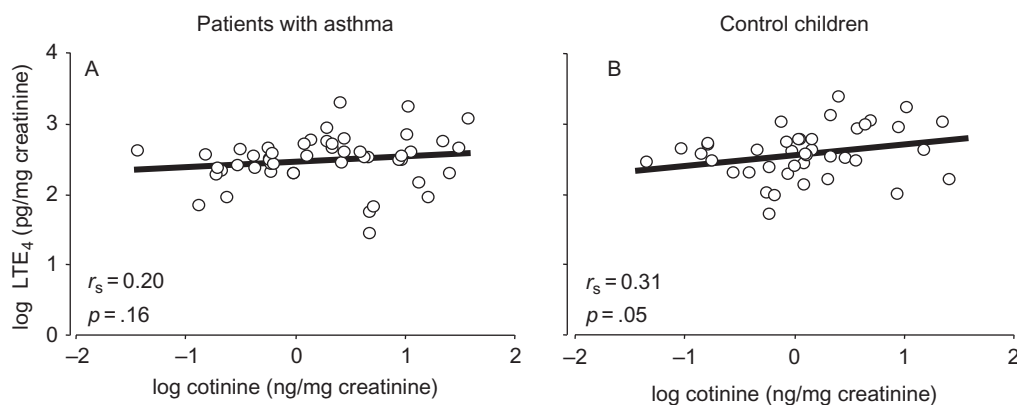


FIGURE 1.—Relationship between log-transformed urinary concentrations of cotinine and LTE_4 . Symbols represent school-aged children with (A) or without (B) mild stable asthma and with different levels of exposure to environmental tobacco smoke. The Spearman correlation coefficient (r_s) and its statistical significance (p) are shown in each panel.

TABLE 3.—Results of multiple linear regression analyses.

Outcome variable	Predictor variables	β coefficient	Standardized β coefficient	p
LTE_4	Cotinine	0.137	0.262	0.11
	BMI	0.004	0.334	0.005
	Cotinine $\times$ Asthma ^a	-0.085	-0.128	0.44
	BMI $\times$ Asthma ^a	-0.001	-0.139	0.25
FEV_1	Cotinine	-5.877	-0.266	0.01
	BMI	0.062	0.117	0.29
	LTE_4	8.336	0.188	0.09
FVC	Cotinine	-6.419	-0.305	0.005
	BMI	0.085	0.167	0.13
	LTE_4	6.569	0.156	0.15
FEV_1/FVC	Cotinine	0.307	0.024	0.83
	BMI	-0.018	-0.060	0.61
	LTE_4	1.735	0.069	0.56

Note: ^aInteraction term introduced in the regression model to evaluate the influence of asthma on the relationship between LTE_4 and cotinine or BMI. BMI, body mass index; FEV_1 , forced expiratory volume at one second; and FVC, forced vital capacity. Units correspond to log pg/mg creatinine (LTE_4), ng/mg creatinine (cotinine), percentile for age (BMI), and % predicted (FEV_1 , FVC, and FEV_1/FVC).

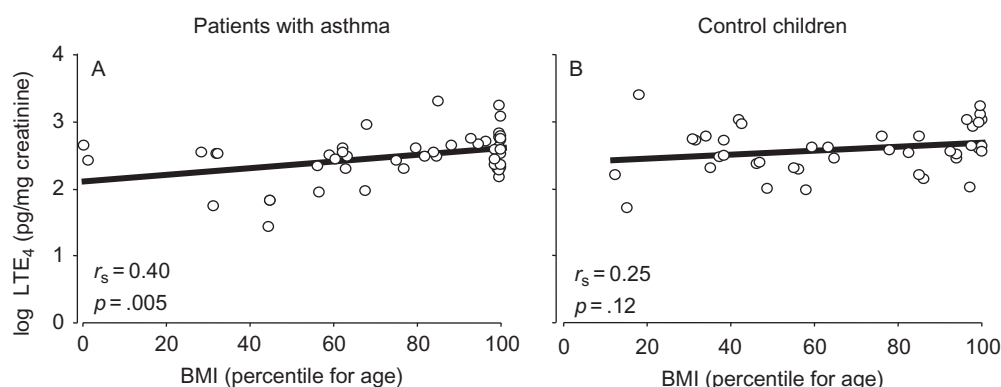


FIGURE 2.—Relationship between body mass index (BMI) and log-transformed urinary concentrations of LTE_4 . Symbols represent school-aged children with (A) or without (B) mild stable asthma. The Spearman correlation coefficient (r_s) and its statistical significance (p) are shown in each panel.

not in the control group (Figure 3). Multivariate analyses corroborated that cotinine exerted more influence on these spirometric parameters than BMI and LTE_4 (Table 3).

DISCUSSION

Exposure to environmental tobacco smoke causes many health consequences, and specifically in subjects with asthma this exposure is associated with a more severe

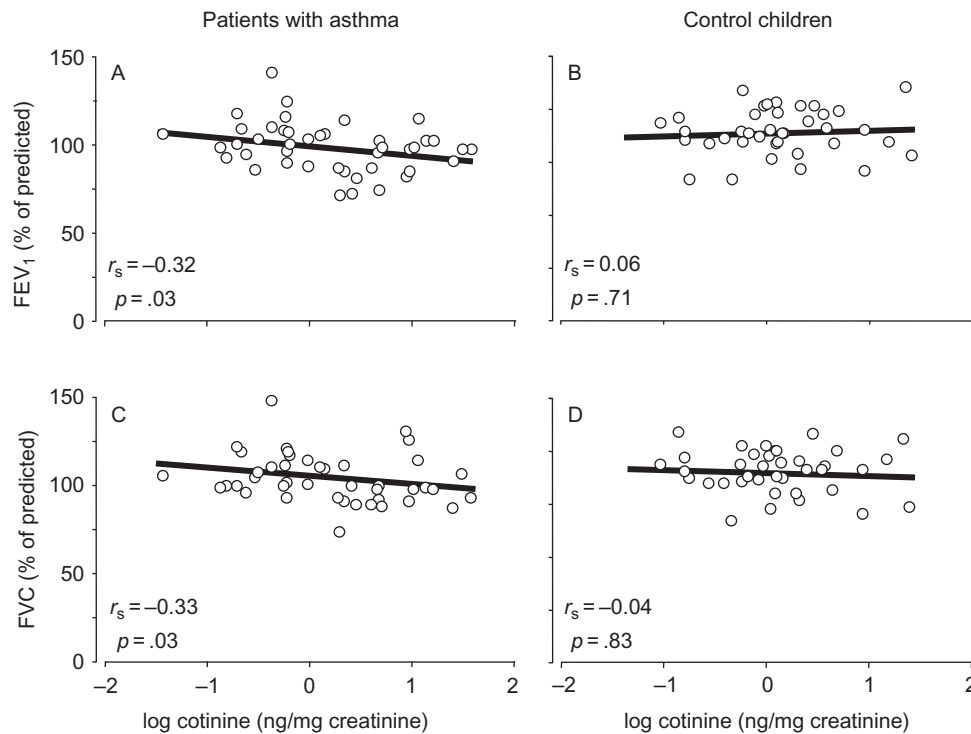


FIGURE 3.—Correlation of urinary concentrations of cotinine with lung function. Symbols represent school-aged children with (A) or without (B) mild stable asthma and with different levels of exposure to environmental tobacco smoke. The Spearman correlation coefficient (r_s) and its statistical significance (p) are shown in each panel. FEV₁ = forced expiratory volume in one second, FVC = forced vital capacity.

disease course and a higher frequency of exacerbations (4, 5). The mechanisms leading to this noxious effect of tobacco are largely unknown. Since their discovery in the 1970s, cysteinyl leukotrienes LTC₄, LTD₄, and LTE₄ attracted much attention due to their possible participation in asthma. This was justified because their biological effects included the majority of the pathophysiological conditions occurring in asthma, such as airway smooth muscle contraction, enhanced vascular permeability, and increased mucus secretion (14). Later, a significant increase of the plasma concentration or urinary excretion of LTE₄ (the stable metabolite of the cysteinyl leukotrienes pathway) was demonstrated during asthmatic exacerbations or inhaled exposure to allergens (15, 16). A role of leukotrienes in asthma was corroborated because drugs that antagonize their major receptor (CysLT1) were effective anti-asthma medications currently recommended by international guidelines for the long-term control of asthma (10). Thus, a potential mechanism to explain the negative impact of passive smoking on asthma is through tobacco-induced leukotriene production. Supporting this possibility is the finding that adult subjects who actively smoke have increased concentrations of urinary LTE₄ (6, 7). Likewise, recent studies in children with asthma indicated a relationship between passive smoking or LTE₄ levels and loss of asthma control (8, 17).

In the present study, we found that urinary excretion of LTE₄ correlated with an objective marker of exposure to tobacco smoke, namely urinary cotinine; however, contrary to what was expected, when both groups were

evaluated separately, there was no significant difference in the cotinine-LTE₄ relationship between patients with and without asthma. If tobacco smoke was acting through direct stimulation of major leukotriene-producing cells, such as mast cells, basophils, eosinophils, or macrophages (18, 19), then the association between LTE₄ and cotinine should have been observed preferentially among children with asthma because these cells are greater in number and possess higher levels of activation in asthmatic airways. Moreover, at least in alveolar macrophages, tobacco products exert an inhibitory effect on the synthesis of lipoxygenase-derived eicosanoids (LTB₄ and 5-HETE) (20, 21). Hence, a direct mechanism triggered by tobacco in these cells appears unlikely, and tobacco smoke may be acting through indirect mechanisms. In this respect, airway epithelium is the first barrier exposed to tobacco smoke, and *in vitro* studies have determined that tobacco smoke or its extracts induce the release of several mediators from airway epithelial cells, some of these with chemotactic and cell-activating properties (22), and it is conceivable that in a healthy epithelium this effect would be stronger than on a damaged one, as occurs in asthma. It is well known that even in mild asthma, the airway epithelial layer is disrupted and partially lost (23). Cells from this injured structure differ from a healthy epithelium with respect to the profile of genes silenced or expressed, probably through epigenetic mechanisms such as DNA methylation (24) and miRNA (25). As a result, differences in the type and amount of cytokines released from asthmatic and normal epithelium are expected to occur (26), either under

baseline conditions or after stimulation with tobacco products. Ultimately, some tobacco-induced cytokines primarily produced in a healthy epithelium may stimulate the leukotriene-producing cells to increase their synthesis and release.

Our study showed that BMI closely correlated with urinary excretion of LTE_4 and that this association was even stronger than that between cotinine and LTE_4 . This is in agreement with two previous studies conducted within another context (sleep apnea/hypopnea obstructive syndrome) that also found a positive association between BMI and urinary LTE_4 (27, 28). It is known that increased numbers of cells capable of releasing cysteinyl leukotrienes, such as M1-macrophages and mast cells, are present in the white adipose tissue of individuals with obesity (29, 30), and it was recently discovered that fat tissue *per se* (adipocytes and stromal vascular cells) can produce and release leukotrienes (31). Thus, production of cysteinyl leukotrienes by fat tissue might easily account for the association between BMI and LTE_4 . Our result on this issue may partially explain why asthmatic patients with overweight or obesity continue to exhibit a clinical response to anti-leukotrienes despite lowered responses to corticosteroids or long-acting beta-adrenergic drugs (32).

It is noteworthy that some children whose parents denied exposure to environmental tobacco smoke did indeed have detectable cotinine levels in urine, and contrariwise, some children supposedly exposed to tobacco smoke had absent or low levels of cotinine (data not shown). These inconsistent results between degree of tobacco exposure by questionnaire and cotinine levels have been already reported by others (33, 34). This might be due to the fact that cotinine is a relatively short-lived metabolite (half-life of 12.8 to 18.8 h) (35); thus, its urinary levels could be absent or low in a putatively passive smoking child if he or she was not sufficiently exposed to tobacco smoke in the hours immediately prior to the urine sample being taken. However, it is interesting to note that even in this context, cotinine correlated with LTE_4 levels, which may imply that the impact of passive smoking on leukotriene synthesis is equally rapid and transient.

Urinary cotinine was negatively associated with lung function, and this tendency was more noticeable in children with asthma than in the control population. This may suggest that asthmatic airways are more susceptible to the harmful effect of tobacco smoke, as has been demonstrated by cross-sectional and longitudinal studies in adults (36). In contrast, cysteinyl leukotrienes are potent constrictors of airway smooth muscle and some reports have described an inverse relationship between urinary LTE_4 and lung function in patients with asthma (37). Nevertheless, other studies have been unable to corroborate such an association in adult asthmatic patients with or without active smoking (38), or in asthmatic children submitted to an exercise challenge test (39). Our results agree with these later reports, inasmuch as urinary LTE_4 did not correlate with FEV_1 or FVC in patients with asthma or in control children. Therefore, it is possible that the more important role

of leukotrienes in asthma is the generation of airway hyperresponsiveness (40, 41), rather than causing airway obstruction.

A potential limitation of our study was that only children with mild, intermittent asthma were included; thus, it is uncertain to what extent our conclusions can be applicable to children with more severe asthma.

In conclusion, in the present study, we found that the urinary levels of LTE_4 were progressively higher as exposure to environmental tobacco smoke increased. However, we found that BMI also increased LTE_4 levels, and this association was even stronger than that with cotinine itself. Finally, urinary cotinine was associated with decreased lung function in children with asthma, but not in healthy children.

DECLARATION OF INTEREST

The authors declare no conflicts of interest related to the content of the manuscript.

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