

The relation between obesity and asthmatic airway inflammation

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Epidemiologic studies suggest increased asthma prevalence in obese subjects. However, the relation between obesity and airway inflammation remains unclear. This cross-sectional study aims to investigate the relation between obesity indices and exhaled nitric oxide (ENO) and leukotriene B₄ (LTB₄) in children with asthma. Asthmatic patients aged 7–18 yr old were recruited. Weight-for-height Z score was calculated from anthropometry. ENO was measured by online single-breath method using a chemiluminescence analyzer, whereas LTB₄ concentrations in exhaled breath condensate (EBC) were quantified using competitive enzyme immunoassay. Ninety-two asthmatics and 23 controls were recruited. The mean ENO and LTB₄ concentrations in EBC were higher in asthmatic patients (87 p.p.b. and 40.5 pg/ml) than controls (25 p.p.b. and 18.7 pg/ml) ($p < 0.0001$ for both). Obesity, as defined by weight $> 120\%$ median weight-for-height, was not associated with any alteration in ENO or LTB₄ concentrations in patients with asthma. Besides, these inflammatory markers did not differ between asthmatics in the highest and lowest quartiles of weight-for-height Z score. On multivariate analysis, ENO showed significant correlation with age ($\beta = 0.511$, $p < 0.0001$), peripheral blood eosinophil count ($\beta = 0.222$, $p = 0.019$), plasma total IgE concentration ($\beta = 0.187$, $p = 0.050$) and forced expiratory volume in 1-s (FEV₁; $\beta = -0.221$, $p = 0.014$). None of the factors was associated with LTB₄ concentration in EBC. In conclusion, ENO and LTB₄ concentration in EBC are increased in childhood asthma. However, these inflammatory markers did not differ between obese and non-obese children with asthma.

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Asthma is one of the most common chronic illnesses in children (1). The simultaneous rise in the prevalence of asthma and obesity (2) has prompted investigators to speculate that obesity might be a causal factor in the development of asthma. Several cross-sectional studies found obesity to be associated with asthma diagnosis, respiratory symptoms and airway hyper-responsiveness (3–5). In the Third National Health and Nutrition Examination Survey, a significant positive association was observed between body mass index (BMI) and asthma but not atopy or eosinophil counts (6). Mechanical properties of the respiratory system associated with obesity may account for these results (7). On the contrary, recent literature suggests that obesity

is a proinflammatory state (8). Increased levels of leptin and proinflammatory cytokines such as interleukin (IL)-6 and IL-1 in obese subjects may up-regulate inflammation in the airway (8). There has not been any data on whether obese asthmatics suffer from more intense airway inflammation.

Infiltration by eosinophils, mast cells and T lymphocytes is a key feature of airway inflammation in asthma (9). Increased eosinophil numbers in induced sputum of asthmatic children are linked to current asthma symptoms and airway hyper-responsiveness (10). Recently, exhaled nitric oxide (ENO) has been shown to have good correlation with airway eosinophilia in bronchial biopsies (11), bronchoalveolar

lavage (12) and induced sputum (13) in children with asthma. ENO also appeared to be sensitive to inhaled corticosteroid (ICS) treatment (14, 15). In addition to eosinophilic inflammation, there is increasing evidence that neutrophils may play a role in more severe asthma (16). Airway inflammation during acute exacerbations of childhood asthma is characterized by infiltration and activation of both eosinophils and neutrophils (17). Increased levels of leukotriene B₄ (LTB₄), released by neutrophils, were found in children with acute asthma exacerbation and up to 1 month after recovery (18). Csoma et al. found LTB₄ to be increased in exhaled breath condensate (EBC) from children with asthma (19). In contrary to ENO, neutrophilic airway inflammation mediated by LTB₄ might not respond to ICS (20, 21). Thus, LTB₄ measurement may supplement ENO in providing useful information on the extent of airway inflammation.

There has not been any report that studied the relation between obesity indices and ENO or exhaled breath markers in asthmatic patients. From the various epidemiologic studies (3–6), we hypothesize that obese asthmatic children have more severe inflammation in their lower airways. This study aims at investigating whether obese children with asthma have increased airway inflammation as measured by ENO and LTB₄ concentration in EBC.

Materials and methods

Study population

Patients aged 7–18 yr with asthma diagnosed according to the American Thoracic Society (ATS) criteria (22) were recruited from the pediatric clinics of a university teaching hospital. Patients were classified into intermittent asthma (IA) and persistent asthma (PA) according to the Global Initiative for Asthma guidelines (23). Prophylactic treatments for asthma remained unchanged for at least 3 months before study. Asthmatics did not have any disease exacerbation for 4 wk before recruitment. Non-allergic schoolchildren who participated in the International Study of Asthma and Allergies in Childhood (ISAAC) (24) were recruited as the comparison group. All subjects were free from respiratory tract infection for 2 wk prior to study. They were examined for any sign of puberty during the study visit. Subjects or their parents gave informed written consent, and the Clinical Research Ethics Committee of our university approved this study.

Anthropometric measurement

Body weight and height were measured with the subject lightly clothed and bare-footed using an electronic weighing scale (Model 708, Seca, Germany) and Harpenden stadiometer. According to World Health Organization, obesity is defined as having weight more than 120% of the median weight-for-height (25). Weight-for-height Z score was also used to compare any difference in inflammatory markers between subjects in the highest and lowest quartiles (25). Weight-for-height Z score was calculated as follows: weight-for-height Z score = (measured weight – expected mean weight for subject's height and sex)/expected standard deviation in weight for subject's height and sex.

Spirometry

All subjects underwent spirometric assessment (Model 2130, Vitalograph, Buckingham, England) to measure FEV₁ and FVC, and results were compared with local age- and sex-matched predicted values (26).

Measurement of exhaled nitric oxide concentration

The ENO was measured at an expiratory flow rate of 50 ml/s using a chemiluminescence analyzer (NOA280i, Sievers Instruments, Boulder, CO, USA) according to ATS standard (27). Repeated exhalations were performed without nose clip until three NO plateau values agreed at the 10% level or two agreed at the 5% level. The mean ENO value was then recorded.

Collection of exhaled breath condensate

The EBC was collected using a disposable collection kit (RTube; Respiratory Research, Inc., Charlottesville, VA, USA) according to manufacturer's instructions. After rinsing their mouth, the recruited subjects breathed tidally for 10 min through a microbial filter (0.3 µm) and a mouthpiece that was connected through a unique one-way valve into a cooled collection tube (28). This EBC collection device includes a trap to exclude saliva contamination. EBC collected along the wall of the tube was stored immediately at –70°C until analysis.

Measurement of LTB₄ concentration

The EBC samples stored at –70°C were analyzed in duplicates for LTB₄ concentrations in a batch using acetylcholinesterase competitive enzyme

immunoassay (Cayman Chemical, Ann Arbor, MI, USA). The intra-assay and inter-assay variability were <10%, and the detection limit of this assay was 4 pg/ml.

Atopy-related markers in peripheral blood

Total immunoglobulin E (IgE) concentrations in ethylenediaminetetraacetic acid (EDTA) plasma were measured by microparticle immunoassay (IMx analyser, Abbott Laboratories, Abbott Park, IL, USA), and analyzed following logarithmic transformation ($\text{IgE}_{\log}$). Eosinophils in peripheral blood (PB) were enumerated in the asthmatic patients using a Coulter STKS counter (Beckman-Coulter, Miami, FL, USA).

Statistical analysis

Data was presented in mean and s.d. unless stated otherwise. Results were analyzed two-sided by Student's t-test or ANOVA for parametric variables and Mann-Whitney U-test or Kruskal-Wallis test for non-parametric numerical variables. Chi-square was used to compare proportions. Comparison for ENO and LTB_4 concentrations between patients in the highest and lowest quartiles of weight-for-height Z score was made using Student's t-test. The correlations between ENO and LTB_4 concentrations in EBC among asthmatic patients and their age, anthropometric data (weight, height, BMI, and weight-for-height Z score), laboratory parameters (PB eosinophil count, and $\text{IgE}_{\log}$) and spirometric variables (FEV_1 , FVC, and FEV_1/FVC) were determined by Pearson correlation. Multivariate stepwise linear regression was then

used to delineate the factors independently associated with ENO and LTB_4 concentrations, with coefficients of the regression lines being expressed as β -values. The p-values <0.05 were considered statistically significant.

Results

Demographic data

Ninety-two asthmatic children and 23 controls were recruited, and their mean (s.d.) age were 12.7 (3.4) yr and 13.6 (0.4) yr respectively ($p = 0.203$). The clinical characteristics of these subjects are summarized in Table 1. Although controls had significantly higher weight and height, obesity indices including BMI and weight-for-height Z score did not differ among the subject groups. Patients with PA and IA were matched as to their demographic, anthropometric, spirometric, and laboratory parameters.

Patient factors and airway inflammatory markers

The mean (s.d.) ENO concentrations in 69 pubertal and 23 pre-pubertal asthmatic children were 101 (68) p.p.b. and 44 (33) p.p.b. respectively ($p = 0.0002$). On the contrary, LTB_4 concentrations in EBC did not differ between the two groups ($p = 0.932$). These two inflammatory markers were also similar among male and female asthmatic patients ($p = 0.154$ and 0.721 respectively).

Asthma and ENO and LTB_4 concentrations

The mean (s.d.) ENO concentrations in patients with PA, IA and controls were 84 (69) p.p.b., 91

Table 1. Clinical characteristics of our subjects

Characteristics*	PA (n = 53)	IA (n = 39)	Controls (n = 23)	p-value
Age (yr)	12.7 $\pm$ 3.4	12.7 $\pm$ 3.5	13.6 $\pm$ 0.4	0.444
Male gender, n (%)	36 (68)	28 (72)	14 (61)	0.673
Body mass index (kg/m^2)	18.5 $\pm$ 3.6	19.3 $\pm$ 3.6	19.1 $\pm$ 2.9	0.546
Weight-for-height Z score	0.20 $\pm$ 1.13	0.36 $\pm$ 1.02	-0.12 $\pm$ 0.88	0.215
Spirometric assessment†				
FEV ₁ predicted (%)	88 $\pm$ 19	82 $\pm$ 13	85 $\pm$ 13	0.341
FVC predicted (%)	101 $\pm$ 20	95 $\pm$ 17	96 $\pm$ 12	0.289
FEV ₁ /FVC (%)	80 $\pm$ 11	79 $\pm$ 10	83 $\pm$ 11	0.372
Laboratory markers				
PB eosinophils (%)	8.0 $\pm$ 3.6	7.7 $\pm$ 3.7	ND	0.784
PB eosinophils ($\times 10^3/\text{l}$)	0.56 $\pm$ 0.28	0.53 $\pm$ 0.27	ND	0.635
$\text{IgE}_{\log}$ in (kIU/l)	2.75 $\pm$ 0.55	2.70 $\pm$ 0.56	1.73 $\pm$ 0.44	<0.001
Current ICS use, n (%)	53 (100)‡	0	0	NA

IA, intermittent asthma; ICS, inhaled corticosteroid; $\text{IgE}_{\log}$, log-transformed plasma total IgE concentration; NA, not applicable; ND, not done; PA, persistent asthma; PB, peripheral blood.

*Expressed as mean $\pm$ s.d. unless otherwise stated.

†Acceptable spirometry was obtained for 50 (94%) subjects with PA, 30 (77%) subjects with IA and all 23 controls.

‡The median (range) daily dose was 250 (50–2000) μg of beclomethasone dipropionate or equivalent.

(62) p.p.b. and 25 (12) p.p.b. respectively ($p = 0.0002$ for PA vs. control and $p < 0.0001$ for IA vs. control). Likewise, LTB_4 concentrations in EBC were increased in patients with PA (mean $\pm$ s.d.: 38.6 ± 14.9 pg/ml) and IA (43.0 ± 11.1 pg/ml) when compared with controls (18.7 ± 9.3 pg/ml; $p < 0.0001$ for both). Nonetheless, neither ENO nor LTB_4 concentration differed between patients with PA (i.e., with the use of ICS) and IA (i.e., steroid-naïve).

Obesity indices and ENO and LTB_4 concentrations

Twenty-two (24%) asthmatic children were classified as being obese (i.e., weight $> 120\%$ median weight-for-height). The mean (s.d.) ENO in obese and non-obese patients were 81 (58) p.p.b. and 89 (69) p.p.b. ($p = 0.597$), and the mean (s.d.) LTB_4 concentrations in EBC from these two groups were 41.8 (8.5) pg/ml and 40.0 (14.8) pg/ml respectively ($p = 0.591$). The distribution of ENO and LTB_4 concentrations among patients in the four quartiles of weight-for-height Z score are illustrated in Figs 1 and 2. These two airway inflammatory markers did not differ between patients in the highest and lowest quartiles. The results on ENO and LTB_4 concentrations between obese and non-obese patients remained insignificant when statistical analyses were repeated in patients with either PA or IA alone (results not shown). Among 23 controls, ENO and LTB_4 levels in EBC did not differ among subjects in the four quartiles of weight-for-height Z score (p for trend = 0.114 and 0.871 respectively).

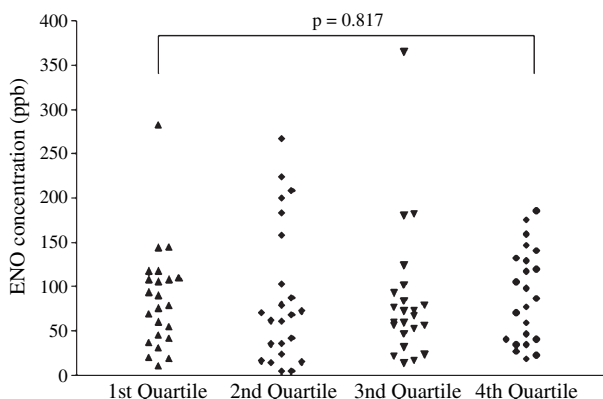


Fig. 1. The distribution of exhaled nitric oxide (ENO) among asthmatic patients in the four quartiles of weight-for-height Z score. The mean values for these four quartiles were 86, 88, 84 and 89 p.p.b., respectively.

Factors affecting ENO and LTB_4 concentrations

The correlations among ENO and LTB_4 concentrations and different demographic, anthropometric, laboratory, and spirometric factors are described in Table 2. Neither BMI nor weight-for-height Z score was associated with ENO concentration. Following linear regression, age ($\beta = 0.511$, $p < 0.0001$), PB eosinophil percentage ($\beta = 0.222$, $p = 0.019$), $\text{IgE}_{\log}$ ($\beta = 0.187$, $p = 0.050$) and FEV_1 ($\beta = -0.221$, $p = 0.014$) showed significant correlation with ENO (Table 3). Subgroup analysis revealed different factors influencing ENO in asthmatic patients < 12 and ≥ 12 yr old. In the former group

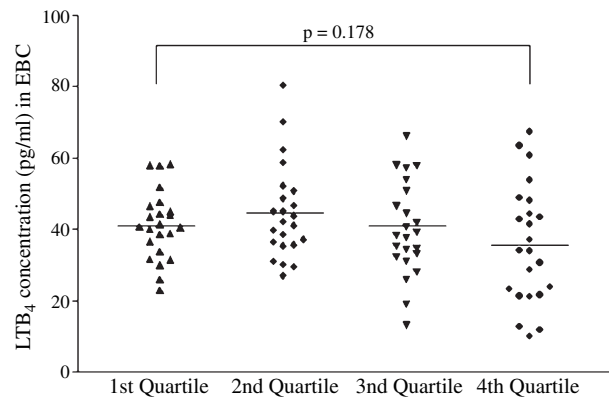


Fig. 2. The distribution of leukotriene B_4 (LTB_4) concentrations in exhaled breath condensate (EBC) among asthmatic patients in the four quartiles of weight-for-height Z score. The mean values (horizontal bars) for these four quartiles were 41.3, 44.7, 40.0, and 35.8 pg/ml, respectively.

Table 2. Univariate analysis of factors potentially associated with ENO and LTB_4 concentrations among our 92 asthmatic patients

Factors	ENO		LTB_4 in EBC	
	Correlation	p-Value	Correlation	p-Value
Age	0.550	< 0.0001	0.019	0.854
Weight	0.409	< 0.0001	0.062	0.554
Height	0.511	< 0.0001	-0.031	0.766
Body mass index	0.203	0.052	0.142	0.176
Weight-for-height Z score	0.007	0.944	0.167	0.112
Laboratory markers				
PB eosinophil percentage	0.306	0.004	-0.100	0.355
PB eosinophil number	0.177	0.100	-0.138	0.199
$\text{IgE}_{\log}$	0.361	0.0004	-0.091	0.390
Spirometric variables				
FEV_1 predicted	-0.280	0.012	-0.018	0.873
FVC predicted	-0.245	0.029	-0.173	0.125
FEV_1/FVC	-0.147	0.192	0.197	0.079
Exhaled NO level	NA	NA	-0.014	0.896
LTB_4 levels in EBC	-0.014	0.896	NA	NA

EBC, exhaled breath condensate; FEV_1 , forced expiratory volume in 1 s; FVC, forced vital capacity; $\text{IgE}_{\log}$, log-transformed plasma total IgE concentration; NA, not applicable; PB, peripheral blood; ENO, exhaled nitric oxide; LTB_4 , leukotriene B_4 .

Table 3. Multivariate linear regression analysis of the factors affecting ENO in our asthmatic patients

Factors	β -Coefficients	p-Value
Age	0.511	<0.0001
Weight	0.131	0.278
Height	0.228	0.118
Puberty*	-0.088	0.416
Laboratory markers		
PB eosinophil percentage	0.222	0.019
IgE _{log}	0.187	0.050
Spirometric variables		
FEV ₁ predicted	-0.221	0.014
FVC predicted	0.047	0.711

FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; IgE_{log}, log-transformed plasma total IgE concentration; PB, peripheral blood; ENO, exhaled nitric oxide.

*Puberty was entered into the stepwise regression model as a dichotomized variable.

($n = 43$), ENO was associated with age ($\beta = 0.394$, $p = 0.001$), PB eosinophil percentage ($\beta = 0.817$, $p = 0.001$) and number ($\beta = -0.522$, $p = 0.022$) and IgE_{log} ($\beta = 0.317$, $p = 0.010$). On the contrary, ENO in patients ≥ 12 yr old ($n = 49$) was associated only with age ($\beta = 0.374$, $p = 0.032$) and percentage FEV₁ ($\beta = -0.444$, $p = 0.011$) but not the atopic factors. None of the factors tested was associated with LTB₄ concentration in EBC in all the 92 patients or following subgroup analysis.

Discussion

The measurement of ENO and LTB₄ in EBC provides complementary information on airway inflammation of our subjects. Our results show that ENO and LTB₄ concentration in EBC may be used to differentiate children with asthma from controls. On the contrary, this study could not detect any difference in these airway inflammatory markers between obese and non-obese children with asthma. Multivariate analysis reveals that ENO was associated with age, atopic factors (PB eosinophils and plasma total IgE concentration) and lung function impairment (FEV₁) in Chinese asthmatic children but not their anthropometric variables. These ENO results were consistent with some of the findings, but not others, in several recent reports (29–31). Besides, ENO in our asthmatics and controls were also significantly higher than the corresponding values in these studies. None of the factors tested in this study shows significant correlation with LTB₄ concentration in EBC.

Our results do not suggest a difference in the extent of airway inflammation, as shown by ENO and LTB₄ in EBC, among obese and non-

obese asthmatics or normal children. Nonetheless, we could not exclude the possibility that airway inflammation in obese subjects may be caused by immune mechanisms that are independent of NO or eicosanoids. A recent study suggested that obesity is a proinflammatory state associated with increased levels of leptin, IL-6, and IL-1. These immunologic changes may result in increased airway inflammation that is independent of an allergic mechanism (8). Because molecules as large as 65 kDa are present in the expired water vapor (28, 32), future studies may also measure inflammatory mediators such as cytokines (IL-1, IL-6, and TNF- α) in EBC in order to better delineate the extent of airway inflammation in obese subjects. Clinicians should also be cautious about the use of high-dose ICS in treating obese children with moderate-to-severe asthma. These obese subjects may be more susceptible to ICS-related metabolic complications such as weight gain, diabetes mellitus, and hypertension. Obese children with PA who are suboptimally controlled should be investigated for the extent of lower airway inflammation before any decision for using high-dose ICS treatment is made.

Lung function impairment in obese asthmatics may be secondary to altered mechanical properties of the respiratory system. Obese adults had diminished tidal lung expansion, and this compromises force fluctuations acting on the airway. Impaired force fluctuations result in greater contractile responses of airway smooth muscle (7), thereby potentially causing airway hyper-responsiveness that is a hallmark of asthma. Li et al., however, found reduction in function residual capacity and impairment of diffusion capacity, rather than obstructive ventilatory changes, to be the predominant features in obese Chinese children (33). Further research should also investigate for any alteration in the mechanical properties of the airway and lung parenchyma in obese asthmatic children.

The assessment of ENO and EBC LTB₄ concentrations in this study provides a reasonably good indication on the extent of airway inflammation. One of the major events in asthmatic inflammation is the infiltration of airways by eosinophils and mast cells (9), and eosinophilic airway inflammation may persist even in asymptomatic asthmatic children with normal lung function (34). ENO showed a good correlation with airway eosinophilia (11–13), and both could be suppressed by the use of ICS (14, 15). On the contrary, LTB₄ is a marker of neutrophilic airway inflammation that is a characteristic feature mainly in patients with poorly controlled

asthma (16–18). LTB₄ appeared to be resistant to ICS immunomodulation (20, 21). Thus, the inclusion of both ENO and LTB₄ measurement enables us to get a clear picture on the severity of airway inflammation in our subjects.

The LTB₄ concentrations measured in EBC of our subjects were much lower than those published previously (19). In that study, Csoma et al. reported that the mean LTB₄ concentrations in EBC were 130 pg/ml for PA, 53 pg/ml for IA and 48 pg/ml for healthy children. These values were significantly higher than the corresponding LTB₄ levels in our subjects despite the fact that both groups used the same immunoassay method (supplied by the same manufacturer) to measure LTB₄. Several reasons may account for this observation. The genetic control over NO and LTB₄ synthesis may differ between white and Chinese subjects. Secondly, the EBC collection devices differ in these studies. Our research group collected EBC in *condensed liquid* at –10°C by a portable and disposable kit (RTube; Respiratory Research, Inc.) whereas Csoma and colleagues collected EBC as *ice* in an electrical refrigerated system (ECoScreen, Jaeger, Hoechberg, Germany) that also operates at –10°C. The latter device may be associated with more drastic freeze-thaw cycles that can affect the molecules thus collected. Besides, the device used in our study incorporated a microbial filter that may screen out some larger molecules such as LTB₄. It is possible that these methodological differences may influence the efficiency or the quality of EBC collected (28). As EBC is an emerging method in the evaluation of airway inflammation, further studies should address on the effects of different sampling devices or protocols on the collected breath condensate.

The small sample size with only 92 asthmatic children is the main limitation of this study in detecting any difference in exhaled markers between obese and non-obese patients. As there has not been any published data on obesity-related ENO changes, it was difficult for us to estimate the required sample size beforehand. The present study revealed that the mean (s.d.) ENO in Chinese asthmatic children was 87 (65) p.p.b. Our sample size of 23 patients in each group with the highest and the lowest quartiles for weight-for-height Z score would only be able to detect a difference of 55 p.p.b. in ENO with 80% power and 95% confidence. If we were to detect an ENO difference of 13 p.p.b. (i.e., 15%) between the two groups, this study would require a total of 800 local asthmatic patients. Nonetheless, the results from this underpowered pilot study can provide some background data on which to plan a

definitive study on the relation between obesity and asthmatic airway inflammation.

The lack of reliable markers for body fat measurement may also affect our results. As there is no widely accepted definition for obesity, we decided to follow the WHO definition by defining obesity as having weight more than 120% of the median weight-for-height (25). This study used BMI as a surrogate for body fat content. Although other methods such as dual energy X-ray absorptiometry and leg-to-leg bioimpedance offer better estimation of body fat content (35), these measurements are tedious and time-consuming and involve exposure to radiation. Thus, we decided to make use of the simple weight-and-height measurements to define obesity. Further studies investigating the definitive relation between airway inflammation and obesity should include some of the above sophisticated tools for body fat measurement.

Another drawback may be the absence of a group of severely obese subjects. Both weight-for-height Z score and BMI were similar between asthmatic patients and controls. Although we showed that ENO and LTB₄ concentration were not associated with obesity indices in asthmatic children, we do not know whether these findings could be extrapolated to extremely obese children in Hong Kong. Nonetheless, the main application of our results is whether the extent of airway inflammation that dictates the dosage of ICS treatment differs between obese and non-obese children with asthma. Thus, future studies should also include a group of severely obese children with asthma.

In conclusion, ENO and LTB₄ concentration in EBC are increased in asthmatic children. However, these airway inflammatory markers did not differ between obese and non-obese children with asthma.

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