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Leptin and exercise-induced bronchoconstriction in obese asthmatic children

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Abstract

Exercise-induced bronchoconstriction (EIB) in asthmatic children has been associated with obesity. Leptin, the proinflammatory adipokine, is typically increased in obesity, whereas cysteinyl leukotrienes (cysLTs) are the main inflammatory mediators implicated in the pathogenesis of EIB. The aim of this work was to study the possible impact of obesity on the severity of EIB in asthmatic children and the possible association with the adipokine leptin. The study included eighty pre-pubertal asthmatic children divided according to their body mass index (BMI) and response to exercise into four groups; obese exercise-responders (n=20), normal-weight exercise-responders (n=20), obese exercise non-responders (n=20), and normal-weight exercise non-responders (n=20). A baseline spirometry test and a standardized exercise challenge test (ECT) were performed. The severity of EIB was assessed by the maximum percentage fall in forced expiratory volume in 1 second ($MF\%FEV_1$) after exercise. The level of fasting serum leptin and the release of cysLTs during exercise were compared between the 4 groups. The $MF\%FEV_1$ during exercise was significantly greater in obese responder compared to normal-weight responder patients ($p=0.004$) and $MF\%FEV_1$ was positively correlated with cysLT release during exercise, BMI z-score, waist circumference and serum leptin. CysLT release during exercise was positively correlated with the level of serum leptin ($r=0.514$, $p=0.001$). This study reports that the severity of EIB is significantly greater in obese compared to normal-weight asthmatic children and suggests an association between leptin and airway hyperresponsiveness to exercise in obese asthmatic children through a mechanism related to cysLT release.

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INTRODUCTION

Exercise-induced bronchoconstriction (EIB) is a clinical condition where a brief period of vigorous exercise or increase in ventilation triggers acute transient lower airway narrowing.[1] It is one of the most common manifestations of asthma, particularly in children and adolescents, occurring in up to 90% of asthmatic children, and in 6 to 20% of the general pediatric population.[2] EIB is highly specific for asthma in children and may precede other asthma symptoms. It can have a detrimental impact on the physical activity of affected children; compromising their participation in play and sports, and negatively influencing their quality of life, cardiovascular condition, and psychomotor development.[3]

The exact mechanism of EIB is not completely understood. However, it's well established now that EIB reflects the degree of airway inflammation and airway hyperresponsiveness (AHR). The airflow limitation, that develops after exercise in susceptible population, is believed to result from hyperosmolar triggering of mast cells and other inflammatory cells within the airways. This leads to the release of bronchoconstrictive mediators like histamine, prostaglandins and cysteinyl leukotrienes (cysLTs).[4]

Several factors were claimed to affect the pathogenesis of EIB in asthmatic patients, among which is obesity. Recent studies[5,6] have reported increased EIB frequency, severity or recovery time among obese patients, and although multiple mechanisms have been proposed, yet a clear cause-effect relationship is still elusive.

Obesity is now recognized as a low-grade inflammatory state and obesity-mediated inflammation has been associated with asthma. The adipose tissue releases a group of proinflammatory adipokines, including leptin, that may be able to enhance airway inflammation and airway hyperresponsiveness.[7] Clinical studies have demonstrated elevated leptin in obese compared to normal-weight asthmatic children,[8] and serum leptin level correlated with lower airway obstruction and EIB among obese asthmatic children.[9] However, the key inflammatory molecules that are involved in this relationship are still to be identified.

A recent study has revealed that exogenous leptin can enhance the production of cysLTs,[10] the lipid inflammatory mediators which are known to play a key role in the pathogenesis of EIB. Therefore, Baek et al. (2013)[11] has suggested a role for leptin-induced LT secretion in the increased prevalence or severity of EIB in obese patients. However, this is still controversial since these results have not been reproduced in other studies.

In view of the potential association between leptin and cysLT production, and their possible effect on the pathogenesis of EIB in obese patients, we hypothesized that high leptin levels in obese asthmatic patients can be associated with an exaggerated release of cysLTs during exercise and ultimately enhance post-exercise bronchoconstriction. The current study was designed to test this hypothesis through the assessment of spirometric and biochemical responses to exercise in relation to anthropometric parameters in asthmatic children.

MATERIALS AND METHODS

STUDY SUBJECTS:

Patients were recruited from asthma outpatient clinic of Alexandria University Children's Hospital, Alexandria, Egypt. Participants were children with stable bronchial asthma. Inclusion criteria were being in the pre-pubertal stage according to Tanner criteria for girls and boys[12, 13] and a physician diagnosis of chronic persistent asthma according to Global Initiative for Asthma (GINA).[14]

Exclusion criteria were unexplained weight change during the past 3 months, inflammatory/endocrine disease, the occurrence of acute asthma exacerbation, respiratory tract infection or any respiratory disorder other than asthma within the previous month. Patients were also excluded if they had used systemic or inhaled corticosteroids, leukotriene modifiers, or long-acting antihistamines during the previous month. Subjects were asked to withhold their short-acting β2-agoinst inhalers for at least 8 h before the exercise challenge and were instructed to avoid caffeine-containing food and drinks and strenuous exercise on the study day.

Legal guardians of the participant children signed an informed consent prior to their inclusion in the study and the study procedures were approved by the Research Ethics Committee of the Medical Research Institute, Alexandria University, Egypt (IORG0008812).

STUDY DESIGN

This study was designed to explore the effect of obesity on the severity of EIB in Egyptian children with bronchial asthma and the possible role of leptin-cysLT interaction. This was sought by comparing the spirometric and biochemical responses to exercise between obese and normal-weight asthmatic children.

Eighty asthmatic children were enrolled to answer the research question. Patients were assigned to one of the following groups according to their body mass index (BMI) and response to ECT: obese exercise-responders (OR) (n = 20), normal-weight exercise-responders (NR) (n = 20), obese exercise non-responders (ON) (n = 20) and normal-weight exercise non-responders (NN) (n = 20).

METHODS

All the study procedures were performed in the Clinical Physiology Unit, Medical Research Institute, Alexandria University, Egypt.

Anthropometric Measurements

All participants underwent detailed history taking and complete physical examination followed by anthropometric measurements including height, weight and waist and hip circumferences. Height and weight were both measured in the quiet standing position, barefoot and wearing light clothing with a (Continental Scale and Stadiometer) to the last complete 0.5 cm and 0.5 kg, respectively.

Body mass index (BMI) was calculated for each patient by dividing the weight (kg) by the square of the height (m^2) and a "z-score" was calculated for each patient. BMI z-scores were compared to normal age- and sex- specific body mass indices (BMIs) according to the World Health Organization (WHO) growth reference for school-aged children and adolescents (2007).[15] Patients with a (+1SD > BMI ≥ -2SD) were assigned to the normal weight group, and those with a BMI ≥ +2SD were assigned to the obese

group. Underweight (BMI < -2SD) and overweight (+1SD ≤ BMI < +2SD) individuals were excluded.

Evaluation of Lung Function

A baseline spirometry test was performed using a MasterScreen™ Pneumo Spirometer (CareFusion, Hoechberg, Germany), with SentrySuite® software and a calibrated Jaeger™ pneumotachograph. Calibration of the spirometer was daily checked, and the measurements were corrected to body temperature (i.e. 37°C), ambient pressure and saturation with water vapor (BTPS). The maneuver was performed and checked for acceptability and repeatability according to the ATS/ERS recommendations.[16]

Every patient completed at least three acceptable maximal forced expiratory maneuvers, and the highest values were automatically recorded. Forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), and forced expiratory flow at 25-75% of FVC ($FEF_{25-75\%}$) were measured and represented as percent of the predicted values (%pred) and the ratio FEV_1/FVC% was calculated according to ATS/ERS criteria.[16]

Exercise Challenge Test (ECT)

An exercise challenge test (ECT) was conducted by running on a motor-driven treadmill with adjustable speed and grade, with the nose clipped, using a standardized protocol.[17] Heart rate was measured at rest and continuously monitored throughout the test using (Acumen heart rate transmitter). The temperature in the laboratory was kept at 22°C and humidity at 40% - 50%.

Starting at a low speed and grade, both were progressively advanced during the first 2 min of exercise until the heart rate reached 85% of the predicted maximum (220 – age in years), then they were maintained for 4 min with a total duration of exercise of 6 min.

Spirometry was repeated serially at 1, 5, 10, 15, 20 and 30 minutes after cessation of the ECT. The response to exercise was monitored by plotting FEV_1, as a percentage of the pre-exercise baseline FEV_1, at each post-exercise interval.

The maximum percentage fall in FEV_1 ($MF\%FEV_1$) was calculated as (Pre-exercise FEV_1 – Lowest post-exercise FEV_1) / Pre-exercise FEV_1 X 100

$MF\%FEV_1$ > 12% predicted was considered as a positive response for diagnosis of EIB[14] and the value of $MF\%FEV_1$ after exercise was used for further analysis as the severity of EIB. An increase of FEV_1 after exercise was noted as a fall of 0% from baseline.

Serum Leptin Level

Blood samples were collected around 8 a.m. after an overnight fast, the blood was centrifuged for 10 min at 5,000 r.p.m. and stored at –70° C. Serum leptin level was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's protocols [Diagnostics Biochem. Canada Inc. (DBC), Ontario, Canada] and expressed as nanograms per milliliter of serum (ng/ml).

Urinary leukotriene E_4 ($U\text{-}LTE_4$)

Pre-exercise urine samples were collected 5 minutes before ECT, then post-exercise urine samples were collected 1 hour after the end of the ECT for assessment of baseline and post-exercise LTE_4 respectively. All urine samples were coded and stored at -70° C until analysis. The levels of LTE_4 in urine before and after ECT were quantified using a commercial ELISA kit (WKEA

Med Supplies Corp, Changchun, China), according to manufacturer's recommendations. To correct for variations in diuresis, creatinine concentration was assessed in urine, and urinary LTE_4 level (U-LTE_4) was expressed as picograms per milligram of creatinine (pg/mg creatinine).

STATISTICAL ANALYSIS

Data were analyzed using IBM SPSS software package version 20.0 (SPSS Inc, Chicago, IL, USA). Continuous data were expressed as means ± SDs. F-test (ANOVA) was used to compare between the four studied groups and Post Hoc test (Tukey) for pair-wise comparisons. When participants were subdivided into two groups (obese vs non-obese), the unpaired student's t-test was used for comparisons. Correlations were calculated using the Pearson Product-Moment correlation test.

Categorical variables were expressed as numbers with corresponding percentages and were analysed using chi-square test. *P* less than 0.05 was considered significant.

RESULTS

Patient Characteristics

Eighty children (age 8.99 ± 1.93 years) with a history of chronic bronchial asthma completed this study. Patients were grouped according to their BMI and bronchial response to exercise into 20 obese exercise-responders (OR), 20 normal-weight exercise-responders (NR), 20 obese exercise non-responders (ON) and 20 normal-weight exercise non-responders (NN).

Patient characteristics are presented in ***Table 1***. Age, gender distribution and asthma severity were not statistically different between the four groups, while BMI z-score, waist circumference (WC) and waist-to-hip ratio (WHR) were significantly greater in obese patients compared to normal-weight patients.

Regarding the baseline spirometric measurements, FEV_1 (%pred), FVC (%pred), FEV1/FVC% and $FEF_{25-75\%}$ (%pred) did not differ significantly between the four groups. Fasting serum leptin was significantly higher in obese compared to normal-weight patients; however, no significant difference was found in fasting leptin level between OR and ON or between NR and NN. Baseline U-LTE_4 levels did not show a statistically significant difference between the four groups.

Response to Exercise Challenge Test (ECT)

The maximum percentage fall in FEV_1 ($MF\%FEV_1$) after ECT was significantly different between the four studied groups ($p < 0.001$). Although the two responder groups (OR and NR) have shown positive response to ECT ($MF\%FEV_1$ >12%), yet the $MF\%FEV_1$ was significantly greater in OR compared to NR patients (p=0.004) indicating more severe bronchoconsrtictive response to exercise in the obese group, ***Table 1***.

Exercise-responders were grouped according to gender and asthma severity, and the $MF\%FEV_1$ was compared between those groups. No difference was found between male and female patients (p= 0.865) or between different grades of asthma severity (p= 0.207), ***Table (2)***.

The release of cysLTs during exercise was assessed by measurement of **post-exercise U-LTE_4** and calculation of **change in U-LTE_4** (the difference between post-exercise and baseline U-LTE_4). The **change in U-LTE_4** after exercise was significantly higher in all exercise-responders (n=40) versus exercise non-responders (n=40) ($p<0.001$), ***Figure (1)***. Among the responder patients, the **change in U-LTE_4** was significantly

higher in obese patients (OR) compared to normal-weight patients (NR), (p = 0.02).

Association between BMI z-score, serum leptin level, change in U-LTE4 after exercise, and MF%FEV1 in exercise responder patients

The relationship between **serum leptin** level and **BMI z-score** in exercise-responder patients (n=40) was explored and revealed a positive correlation (r = 0.936, p<0.001). The release of cysLT during exercise as assessed by **change in U-LTE$_4$** after ECT was positively correlated with **BMI z-score** (r =0.623, p <0.001), **Figure 2A,** and with **serum leptin** level (r =0.514, p=0.001) in exercise responder patients, **Figure 2B**.

The severity of EIB as assessed **MF%FEV$_1$** after exercise was positively associated with **BMI z-score** (p <0.001), **WC** (p= 0.001), serum **leptin level** (p <0.001), and **change in U-LTE$_4$** (p <0.001) among all exercise-responder patients (n=40), ***Table 3***.

Table 1. Characteristics of the four studied groups.

	Obese Responders (n=20)	Normal-weight Responders (n=20)	Obese Non-responders (n=20)	Normal-weight Non-responders (n=20)	P-value
Gender (% male)	13 (65)	12 (60)	14 (70)	11 (55)	0.785
Age (years)	9.22 ± 1.74	8.95 ± 2.04	9.18 ± 1.95	8.63 ± 2.05	0.770
BMI z-score	3.11 ± 0.94[ac]	-0.41 ±0.78 [b]	3.10 ± 0.80	-0.52 ± 1.12	<0.001*
WC (cm)	78.90±11.08[ac]	62.05±5.06 [b]	83.15 ±9.50	60.0 ± 6.66	<0.001*
WHR	0.89 ± 0.05[ac]	0.86 ± 0.02 [b]	0.91 ± 0.08	0.84 ± 0.03	<0.001*
FEV$_1$ (%pred)	92.01 ±11.99	92.83±14.66	94.23±12.84	99.49 ± 8.53	0.218
FVC (%pred)	91.17 ± 9.80	95.34 ± 9.74	93.74 ± 9.22	93.58 ± 6.92	0.537
FEV$_1$/FVC %	83.16 ± 6.87	82.07 ± 8.79 [b]	83.27 ± 7.04	85.84 ± 6.25	0.190
FEF$_{25-75\%}$ (%pred)	76.76 ± 22.79	70.20 ±18.86	80.30 ± 25.0	83.55 ± 18.41	0.243
Leptin (ng/ml)	40.12±11.16[ac]	5.11 ± 2.32 [b]	38.67 ±12.90	5.49 ± 2.69	0.001*
Baseline U-LTE$_4$ (pg/mg creatinine)	44.19 ± 13.26	40.42 ±13.54	42.37 ±14.02	38.21±13.91	0.056
Change in U-LTE$_4$ (pg/mg creatinine)	45.58±14.06[abc]	33.49±18.34[bc]	7.72 ± 7.15	5.40 ± 5.84	<0.001*
MF% FEV$_1$	26.11 ±6.66[abc]	20.67 ± 5.75[bc]	4.78 ± 3.37	3.13 ± 2.14	<0.001*

BMI z-score: body mass index (SD from adjusted mean); **WC:** waist circumference; **WHR:** waist to hip ratio; **FEV$_1$:** forced expiratory volume in 1 second; **FVC:** forced vital capacity; **FEV$_1$/FVC %:** percentage ratio of forced expiratory volume in 1 sec to forced vital capacity; **FEF$_{25-75\%}$:** maximal flow between 25 and 75% of FVC; **% pred:** percent predicted; **ng/ml:** nanogram per milliliter; **U-LTE$_4$:** Urinary leukotriene E$_4$, **pg/mg creatinine:** picograms per milligram of creatinine; **MF% FEV$_1$**: maximal percentage fall in FEV$_1$= (Pre-exercise FEV$_1$ – Lowest post-exercise FEV$_1$)/ Pre-exercise FEV$_1$ X 100.

a: P < 0.05 vs normal-weight responder patients (post hoc comparisons with Tukey test).

b: P < 0.05 vs obese non-responder patients (post hoc comparisons with Tukey test).

c: P < 0.05 vs normal-weight non-responder patients (post hoc comparisons with Tukey test).

*: Indicates statistical significance.

Table 2. Comparison between MF%FEV1 in different gender and asthma severity groups for all responder patients (n = 40).

	MF%FEV$_1$		
	N	**Mean ± SD**	**p**
Gender			
Male	25	23.53 ± 6.84	0.865
Female	15	23.15 ± 6.78	
Asthma Severity			
Mild	14	21.48 ± 6.12	0.207
Moderate	17	25.58 ± 7.41	
Severe	9	22.22 ± 5.69	

MF% FEV1: maximal post-exercise % fall in FEV1 = (Pre-exercise FEV1 – Lowest post-exercise FEV1) / Pre-exercise FEV1 X 100.

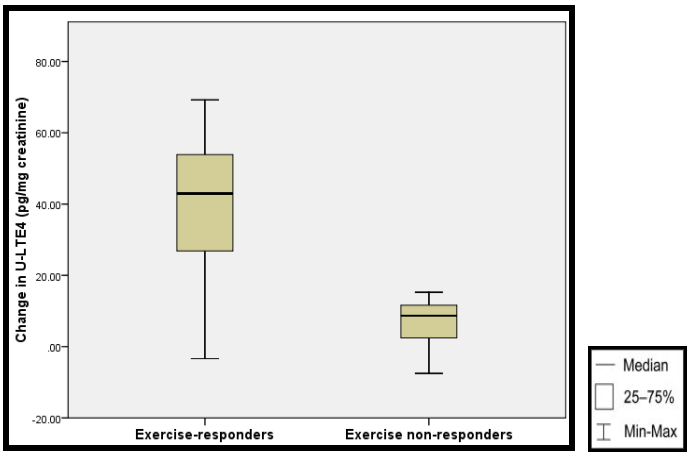

Figure 1. Change in U-LTE$_4$ (pg/ mg creatinine) after exercise challenge test in responder and non-responder patients.

U-LTE4: Urinary leukotriene E4, pg/mg creatinine: picograms per milligram of creatinine

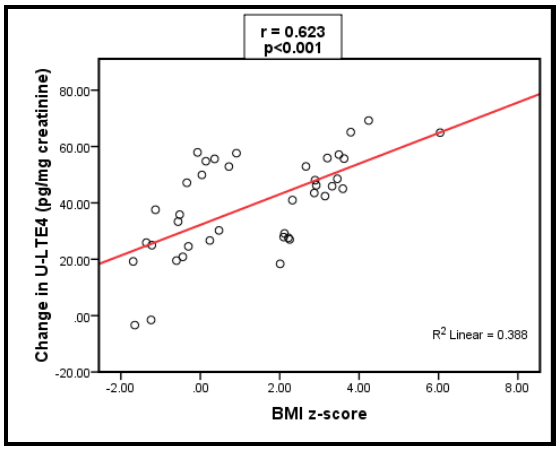

Figure 2. Correlation between BMI z-score and Change in U-LTE$_4$ after exercise in exercise responder patients.

U-LTE$_4$: Urinary leukotriene E$_4$, **pg/mg creatinine:** picograms per milligram of creatinine.

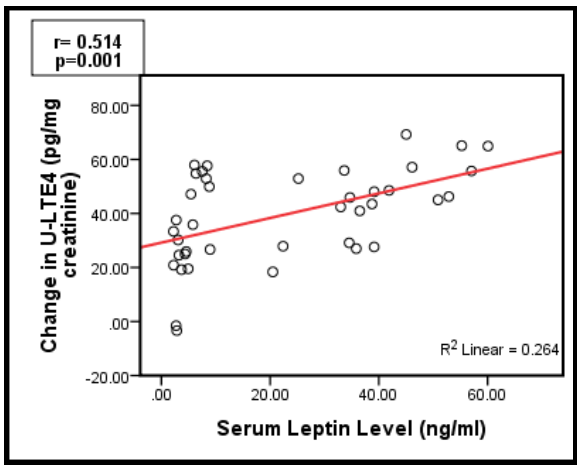

Figure 3. Correlation between Serum leptin level and Change in U-LTE$_4$ after exercise in exercise responder patients.

U-LTE$_4$: Urinary leukotriene E$_4$, **pg/mg creatinine:** picograms per milligram of creatinine.

Table 3. Correlation between MF%FEV$_1$ and different parameters in exercise responder patients (n = 40).

	MF%FEV$_1$	
	r	p
Age (years)	0.165	0.310
Gender	0.028	0.865
Asthma Severity	0.081	0.618
BMI z-score	0.559*	<0.001*
WC (cm)	0.495	0.001*
WHR	0.094	0.564
FEV$_1$ (%pred)	-0.008	0.963
FVC (%pred)	-0.003	0.987
FEV$_1$ / FVC %	-0.002	0.990
MMEF (%pred)	0.012	0.941
Leptin (ng/ml)	0.592*	<0.001*
Baseline U-LTE$_4$ (pg/ mg creatinine)	0.110	0.501
Change in U-LTE$_4$ (pg/ mg creatinine)	0.615*	<0.001*

BMI: body mass index, **WC:** waist circumference, **WHR:** waist to hip ratio, **FVC:** forced vital capacity; **FEV$_1$:** forced expiratory volume in 1 second; **FEV$_1$/FVC:** ratio of forced expiratory volume in 1 sec to forced vital capacity; **MMEF:** maximal mid expiratory flow; **% pred:** percent predicted, **MF% FEV$_1$:** maximal post-exercise % fall in FEV$_1$, **U-LTE$_4$:** Urinary leukotriene E$_4$.

DISCUSSION

The main finding of our study was a greater severity of EIB in obese than in normal-weight asthmatic children, and a positive association between the maximal fall in FEV$_1$ (MF%FEV$_1$) and cysLT release during exercise and the serum leptin level.

The results of our study have shown a greater severity of EIB in obese than in normal-weight asthmatic children and a positive correlation between MF%FEV$_1$ and both the BMI z-score and WC. This finding was in accordance with previous studies which have also reported a greater post-exercise reduction in FEV$_1$ in obese than in non-obese children.[6, 18, 19] In other studies, weight loss was linearly related to a reduction in the prevalence and severity of EIB in obese children and adolescents.[20, 21] In contrast, some studies have reported the absence of a significant association between high BMI and EIB frequency[22] or severity[23]. Rodrigues et al. (2007)[23] have included, in their study, patients with a wide age range: 6-18 years. The hormonal changes, during and after puberty, are known to affect different aspects of body function including airway responsiveness, and hence, in our study, only prepubertal children were included. The authors have also included both overweight and obese patients in one category and this may have affected their results. In addition, they defined EIB on basis of a post-exercise reduction in FEV$_1$ and/or FEF$_{25-75\%}$, and this is, unlike our definition, is not in accordance with the global guidelines.[14]

The effect of obesity on asthma and EIB has been attributed to several mechanisms including the immunomodulatory effects of obesity, mediated by proinflammatory adipocytokines like leptin.[24] Leptin is a protein secreted from white adipocytes that has been implicated in the regulation of energy balance. Its serum level is believed to reflect body fat mass as its secretion is highly correlated to adipocyte size.[25] In the

present study, fasting leptin levels were, in agreement with other studies,[9, 26] higher in obese than in normal-weight children (p <0.001), and linear regression demonstrated a strong positive correlation between BMI and leptin level (p < 0.001). The positive correlation between BMI and leptin was previously reported in Egyptian children by other researchers.[27, 28]

An important finding in our study was the association between leptin and airway response to exercise. Leptin is known to have pro-inflammatory properties as it stimulates the release of pro-inflammatory mediators,[29] and negatively affects the regulatory T cells.[30] Recently, some investigators have also demonstrated that leptin may enhance the Th-2 and ILC_2 responses, thus directly promote allergic airway reactions in asthma.[31] In the current study, the severity of EIB as determined by $MF\%FEV_1$ was positively correlated to fasting serum leptin in asthmatic patients (r=0.592, p<0.001). Some previous studies have suggested that high serum leptin concentrations may affect the severity of asthma, and this was attributed to the enhancing effect of leptin on airway inflammation.[26, 32] Baek et al. (2011)[9] have assessed the serum levels of leptin in asthmatic children and investigated the relation between this hormone and the children's airway response to exercise. Their results, in accordance with ours, confirmed that the $MF\%FEV_1$ after exercise was positively correlated with leptin levels.

Thus, our results and data from literature corroborate the hypothesis that leptin plays a role in inflammatory processes linking obesity and EIB. Yet, many questions regarding the mechanisms involved in leptin-related effects on airway inflammation and EIB remain unresolved. Out of several factors that contribute to chronic inflammation in obese, the 5-lipoxygenase (5-LO) pathway-derived lipid mediators; leukotrienes, have emerged as prominent players.

In the present study, serum leptin levels were significantly associated with the $MF\%FEV_1$ and also with the cysLT release during exercise in exercise-responder patients. These results are in accordance with those of two earlier studies, which also reported a positive association between leptin and U-LTE_4 in asthmatic patients.[11, 33] Our observation that the amount of LT release during exercise, and thus the $MF\%FEV_1$, is significantly associated with serum leptin level may be partially explained by the results of Mancuso et al. (2004)[10] which proved experimentally the ability of leptin to augment LT synthesis in alveolar macrophages.

CysLTs are robust inflammatory mediators that have been proved to play an important role in the pathophysiology of several inflammatory diseases including asthma. [34] The relationship between cysLT and EIB is peculiar as several studies have identified the release of bronchoconstrictive eicosanoids, particularly cysLTs, after exercise as the predominant cause of EIB.[1] However, increased urinary[35] or sputum[36] cysLT levels were detected after exercise in patients with EIB in some studies but not in others.[37]

Recently, a potential link between the LT pathway and adipose tissue (AT) inflammation has been suggested.[38] The expression of the enzyme 5-lipoxygenase (5-LO) and its cofactor 5-LO activating protein (FLAP) was increased in obese AT.[39] In addition, Mothe-Satney et al. (2012)[40]

have proved the secretion of LTs from human adipocytes, and a positive correlation between the LT level and the size of the adipocyte. Thus, as the adipocytes become larger in obesity, the LT levels are increased. They also found that inflammatory stimuli enhanced the LT production in adipocytes, and hence suggested a crucial role for the adipocyte-secreted LTs in the obesity-associated inflammatory reactions. In the present study, we found that the change in U-LTE_4 during exercise was significantly higher in obese than in normal-weight patients and was positively associated with the BMI z-score which suggests a role for cysLTs in the exaggerated EIB response in obese asthmatic children.

Conclusion

In conclusion, our study supports the hypothesis that the severity of EIB is significantly greater in obese compared to normal-weight asthmatic children and suggests an association between leptin and airway hyperresponsiveness to exercise in obese asthmatic children through a mechanism related to cysLT release. However, more studies are needed to explore the cause-effect relationship between leptin and cys-LTs and the role of this relationship in the pathogenesis of EIB.

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