
Serum leptin and adiponectin levels correlate with exercise-induced bronchoconstriction in children with asthma

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Background: Exercise-induced bronchoconstriction (EIB), a form of bronchial hyperresponsiveness (BHR), is common in children with asthma or obesity. Epidemiological studies have shown that asthma and obesity are increasing in parallel, but obesity- and adipokine-related effects on inflammation and BHR have not yet been demonstrated in the human airway.

Objective: To address the relationship between leptin and adiponectin and EIB in children with asthma.

Methods: Eighty-five prepubertal children between the ages of 6 and 10 years were included in our study. They comprised obese with asthma ($n = 19$), normal weight with asthma ($n = 23$), obese without asthma ($n = 23$), and healthy ($n = 20$). We measured serum leptin and adiponectin levels. We also performed pulmonary function tests: baseline, postbronchodilator inhalation, methacholine inhalation, and exercise. The area under the forced expiratory volume in 1 second (FEV₁)–time curve quantified the severity of EIB over a 20-minute period after exercise (AUC₂₀).

Results: The obese children had significantly elevated levels of leptin and reduced levels of adiponectin. The maximum decreases in %FEV₁ and AUC₂₀ after exercise were positively correlated with leptin levels and negatively with serum adiponectin levels in children with asthma. The odds for having EIB were incrementally and significantly higher for children with higher levels of serum leptin.

Conclusions: Levels of the adipocyte-derived hormones leptin and adiponectin are significantly correlated with BHR induced by exercise challenge in children with asthma. Further studies are needed to elucidate whether the changes in leptin and adiponectin levels bear a causal relationship to the EIB/BHR.

Ann Allergy Asthma Immunol. 2011;107:14–21.

INTRODUCTION

Exercise-induced bronchoconstriction (EIB) refers to a condition in which vigorous physical exertion triggers acute airway narrowing in individuals with heightened bronchial hyperresponsiveness (BHR). Exercise-induced bronchoconstriction occurs in approximately half of children with asthma.¹

Epidemiological studies have shown that the prevalence of asthma is increasing concomitantly with obesity.² Emerging data suggest a link between asthma and obesity³ and indicate that the mechanical, immunological, hormonal and inflammatory effects of obesity play a role in the development and persistence of asthma.^{2,4–6} Adipose tissue releases a variety of proinflammatory adipokines, including leptin, adiponectin, interleukin (IL) 6, tumor necrosis factor alpha (TNF- α), plasminogen activator inhibitor 1, eotaxin, vascular endothelial growth factor, and monocyte chemoattractant protein (MCP)-1.^{3,7} Studies in mouse models^{8–11} have demonstrated that obesity

and adipokines enhance BHR, airway inflammation, and allergic responses. However, obesity- and adipokine-related effects on inflammation and BHR have not yet been demonstrated in the human airway. The aim of this study was to address the relationship between the adipocyte-derived hormones leptin and adiponectin and EIB in children with asthma.

METHODS

Subjects and Protocol

The subjects were recruited from the outpatient clinics in Hanyang University Hospital, Korea. Study participants were 85 prepubertal children between the ages of 6 and 10 years who were classified into one of the following groups: obese subject with asthma ($n = 19$), normal-weight subject with asthma ($n = 23$), obese subject without asthma ($n = 23$), and healthy ($n = 20$). Age- and sex-specific body mass index (BMI) percentiles were calculated based on raw height and weight according to the current guidelines of the U.S. Centers for Disease Control.¹² Participants with a BMI below the 85th percentile were categorized as “normal weight,” and those with a BMI at or above the 95th percentile were categorized as “obese.” Underweight individuals below the 5th percentile were excluded from the categorical analysis.

Asthma was defined as the presence of symptoms with an increase in FEV₁ of 12% or more after bronchodilator treat-

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Funding Sources: Self-funded.

Disclosures: The authors have nothing to disclose.

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doi:10.1016/j.anai.2011.03.013

ment, or if less than 16.0 mg/mL of inhaled methacholine induced a 20% decrease in FEV₁ (PC₂₀).¹³ An exercise bronchoprovocation test was used for EIB diagnosis, which was considered positive with a 15% or greater decrease in FEV₁ after exercise.¹³ Classification of asthma severity was based on the guidelines of the Global Initiative for Asthma, using an algorithm including medication dosing, FEV₁, medication adherence, and symptom levels.¹⁴ Control participants were age- and sex-matched healthy children who had no history of wheezing, recurrent or chronic diseases, or infection during the preceding 2 weeks. Exclusion criteria included acute exacerbation of asthma within the previous 6 months and parenchymal lung disease apparent on chest radiography within 4 weeks. Participants were excluded if they had used an inhaled corticosteroid, leukotriene modifier, long-acting antihistamine, or long-acting β_2 -agonist in the 30 days before the study, and those with an FEV₁ below 70% of the predicted value. In the groups without asthma, those with any likelihood of atopy or subclinical airway disease were also excluded by the following criteria: exhaled nitric oxide (eNO) of 30 parts per billion (ppb) or greater; positive skin prick test (SPT) to 1 or more common allergen; or positive methacholine challenge for BHR.¹⁵

All study participants were initially submitted to anthropometric measurements of height and weight made with calibrated equipment. They were also examined for any sign of puberty during the study visit. The SPT for common inhalant and food allergens, including *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, cat dander, dog dander, *Alternaria alternata*, cow milk, egg white, peanut, wheat, and soybean (Allergopharma, Reinbek, Germany) together with negative (saline) and positive (histamine) controls were performed. A positive response was defined as a mean wheal diameter equal to or greater than one half that of the positive control. Fasting blood samples were stored at -70°C until determination of eosinophil cationic protein (ECP), serum total and allergen-specific immunoglobulin (Ig) E, cytokines, leptin, and adiponectin. The eNO levels were measured by a physician. Spirometry, methacholine, and exercise challenge tests were performed by a well-trained technician. All the study procedures were approved by the Medical Ethics Committee of Hanyang University Hospital, and all the subjects gave written informed consent.

Spirometry and Methacholine Challenge

Spirometry and methacholine challenges were conducted in accordance with American Thoracic Society standards.¹³ Spirometry was performed with a Masterlab spirometer (Jaeger Co., Freiburg, Germany).¹⁶ Bronchodilator response was calculated as the percentage change in FEV₁ from baseline 15 minutes after inhaling 0.2 mg salbutamol sulfate (Ventolin, GlaxoSmithKline, Middlesex, United Kingdom) via a metered dose inhaler. Methacholine challenges were performed using a modified Chai procedure.¹⁶

Exercise Challenge

Exercise challenges were conducted in accordance with American Thoracic Society standards.¹³ Exercise challenge testing was performed by running with nose clipped on a treadmill (LE 200 CE, Jaeger Co.), using a standardized protocol. During the test, heart rate was continuously monitored by a radiographic device (electronic electrocardiogram monitor; BCI autocorr). The ambient temperature in the air-conditioned laboratory was kept stable at 22°C, and the humidity was stable between 40% and 50% on each day of the study. Inspired air temperature and humidity were measured. The test started with running at low speed on the treadmill with an incline of 10%. The running speed of the treadmill was increased, raising the heart rate to approximately 85% of the predicted maximum ($[220 - \text{age}] \times 0.9$). This speed was maintained for a maximum of 6 minutes. Spirometry was conducted 20 and 5 minutes before each exercise challenge and repeated 0, 3, 6, 10, 15, and 20 minutes after the end of exercise. The better of at least 2 FEV₁ maneuvers within 5% of each other was recorded at each time point. The area under the FEV₁-time curve (expressed as the percent change from baseline values) over a 20-minute period after exercise (AUC₂₀)¹⁷ was used as an index of the severity of EIB. The AUC₂₀ values were calculated using the trapezoidal rule.

Measurement of Serum Total and Allergen-Specific IgE, and Blood Eosinophil and Eosinophil

Cationic Protein. Serum total IgE, ECP, and specific IgE against the same common allergens that were used for SPT were measured with the Immuno-CAP system (Phadia AB, Uppsala, Sweden). For this analysis, atopy was defined as the presence of at least 1 positive allergen-specific IgE test result (IgE $\geq$ 0.35 kU/L) or SPT finding.

Measurement of Biomarkers

Serum leptin, adiponectin, IL-6, and TNF- α were measured with kits (R&D Systems, Inc., Minneapolis, Minnesota) according to the manufacturer's protocols. Each sample was assayed in triplicate. The lowest limits of detection were 7.8 pg/mL for leptin and TNF- α , 0.246 pg/mL for adiponectin, and 1.6 pg/mL for IL-6.

Exhaled NO

Exhaled NO was measured with a portable nitric oxide analyzer (NIOX MINO, Aerocrine, Solna, Sweden) that provided measurements at a 50 mL/s exhalation flow rate expressed in ppb.¹⁸ Determinations made with the device were in clinically acceptable agreement with measurements provided by a stationary analyzer according to the guidelines of the American Thoracic Society.¹⁹

Statistical Analysis

Data analysis was conducted using SPSS software (version 16.0, SPSS Inc., Chicago, Illinois). Variables measured on a continuous scale were summarized as mean $\pm$ standard deviation. Because of their asymmetric distributions, the levels of cytokines, serum total IgE, PB eosinophil, ECP, and eNO

were reported as interquartile ranges. Comparisons of numerical variables between the 4 study groups categorized by either BMI percentile or diagnosis were evaluated by Kruskal-Wallis tests. Post hoc pairwise comparisons were performed with Tamhane tests to determine differences between groups. Comparisons of categorical variables were evaluated by χ^2 tests or Fisher's exact test. Spearman correlation coefficients were used to assess the associations between pairs of variables measured on a continuous scale. To adjust for BMI, correlations between serum leptin/adiponectin levels and the maximum decreases in FEV₁ and AUC₂₀ were evaluated by partial correlation analysis. Logistic regression models were analyzed to investigate the associations of leptin and adiponectin with EIB.

RESULTS

Characteristics of Study Subjects

The demographic data for the 85 subjects are shown in Table 1. Of the 42 subjects with asthma, 7 had mild intermittent

asthma (3 obese and 4 normal-weight), 14 had mild persistent asthma (7 obese and 7 normal-weight), and 21 had moderate asthma (10 obese and 11 normal-weight) according to the Global Initiative for Asthma guidelines. No significant differences were seen in asthma severity between the obese and normal-weight subjects with asthma. Mean values of age, height, and sex distribution did not differ significantly among the 4 study groups, and no significant differences were seen in rates of atopy, allergic rhinitis, or prior use of inhaled corticosteroids between the obese and normal-weight subjects with asthma.

Pulmonary Function, BHR to Methacholine or Exercise, and Severity of EIB

The parameters of pulmonary function in the study subjects are shown in Table 1. Baseline values of FEV₁, FEV₁/forced vital capacity (FVC), and forced expiratory flow between 25% and 75% (FEF₂₅₋₇₅) in the obese subject with asthma and obese subject without asthma groups were lower than in the

Table 1. Characteristics of the Study Subjects^f

	Subjects with asthma		Subjects without asthma		P ^a
	Obese (n = 19)	Normal weight (n = 23)	Obese (n = 23)	Normal weight (n = 20)	
Age (years)	9.0 (7.0–10.0)	8.0 (6.9–9.3)	9.5 (7.8–10.0)	9.0 (8.1–10.0)	.561
BMI (kg/m ²)	23.0 ± 3.1 ^{b,g}	16.9 ± 2.1	23.3 ± 3.8 ^g	16.8 ± 1.9	<.0001
Sex (% male)	68.4	69.6	56.5	55.0	.663 ^c
Clinical features					
Prior ICS use (%)	47.4	43.5	NA	NA	.801 ^d
Allergic rhinitis (%)	42.1	43.5	NA	NA	.929 ^d
Atopy (%)	84.2	82.6	NA	NA	.267 ^d
Baseline					
FEV ₁ (pred %)	88.2 ± 12.1 ^{g,h}	88.6 ± 12.4 ^g	94.5 ± 16.0 ^g	104.7 ± 11.8	.001
FVC (pred %)	98.2 ± 11.6	97.7 ± 9.2	95.4 ± 9.8	97.5 ± 12.4	.384
FEV ₁ /FVC ratio	82.1 ± 7.7 ^g	85.4 ± 6.5 ^g	85.6 ± 11.6 ^g	92.0 ± 5.3	<.001
FEF ₂₅₋₇₅ (pred %)	68.3 ± 23.2 ^e	74.0 ± 27.2 ^g	81.1 ± 31.9 ^g	92.5 ± 15.0	.043
Postbronchodilator					
ΔFEV ₁ (pred %)	11.2 ± 5.3 ^{g,h}	11.7 ± 5.9 ^g	3.4 ± 6.2	1.9 ± 4.4	<.001
ΔFVC (pred %)	2.7 ± 8.0	2.3 ± 7.1	2.8 ± 5.7	2.2 ± 3.4	.655
ΔFEV ₁ /FVC ratio	8.0 ± 5.0 ^{g,h}	6.5 ± 5.7 ^g	3.4 ± 5.3	3.8 ± 4.9	.008
ΔFEF ₂₅₋₇₅ (pred%)	33.1 ± 17.1 ^{g,h}	33.7 ± 20.8 ^g	7.9 ± 7.2	5.1 ± 8.7	<.001
Postexercise					
EIB (%)	63.2 ^{g,h}	43.5 ^g	13.0	10.0	.232
Maximum decrease in FEV ₁	21.6 (14.2–36.8) ^e	15.0 (11.0–22.8) ^g	9.1 (6.1–11.0)	6.0 (3.3–8.2)	<.0001
AUC ₂₀ (percentage change × min)	432.8 (291.4–719.8) ^e	226.4 (162.2–387.6) ^g	71.8 (56.8–102.0)	39.8 (27.1–59.5)	.018
PC ₂₀ (mg/mL)	6.9 (2.4–12.5)	4.8 (3.3–7.7)	NA	NA	.412 [§]

Abbreviations: BMI, body mass index; ICS, inhaled corticosteroid; NA, not applicable; EIB, exercise-induced bronchoconstriction; PC₂₀, provocative concentration of methacholine inducing a 20% fall in FEV₁; pred %, predicted %.

Data are presented as absolute numbers, mean ± standard deviation, or median (interquartile range).

^a Kruskal-Wallis test.

^b χ^2 test.

^c Fisher exact test.

^d Mann-Whitney U test.

^e Post hoc pairwise comparisons are Tamhane tests.

^f P < .05 versus normal weight subjects with asthma.

^g P < .05 versus healthy.

^h P < .05 versus obese subjects without asthma.

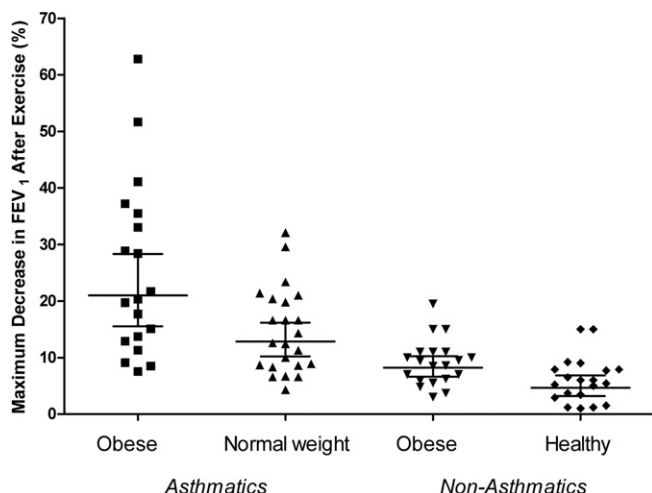


Figure 1. The percentage of maximum decrease in FEV₁ after exercise in the obese subjects with asthma ($n = 19$) and those of normal weight subjects with asthma ($n = 23$), obese subjects without asthma ($n = 23$), and healthy controls ($n = 20$). The horizontal lines in the dot plots represent median and interquartile ranges.

healthy controls. The obese subjects with asthma differed significantly from the obese subjects without asthma in FEV₁ and FEF₂₅₋₇₅ and from the normal-weight subjects with asthma in FEF₂₅₋₇₅. The bronchodilator response of the subjects with asthma was significantly greater than that of the subjects without asthma, as were the EIB frequencies and maximum decrease in FEV₁ after exercise. No further significant differences in EIB frequencies were found between the obese subjects with asthma and normal-weight subjects with asthma. In group comparisons, the maximum decrease in FEV₁ after exercise was significantly greater in the obese subjects with asthma than in the normal weight subjects with asthma (Fig 1), and the same was true of the EIB severity indicator, AUC₂₀ (Table 1). However, after adjusting for both

leptin and adiponectin, neither variable differed significantly between the 2 groups.

Serum Levels of Adipokines, ECP, IgE, and eNO

Biomarker levels are shown in Table 2. Serum leptin levels were significantly higher in both obese subjects with asthma and obese subjects without asthma than in their normal-weight counterparts, and leptin levels were positively correlated with BMI ($r = 0.770$, $P < .001$). In contrast, serum adiponectin was significantly lower in the obese subjects than in their normal-weight counterparts and was negatively correlated with BMI ($r = -0.627$, $P < .001$). No statistically significant differences were seen in serum leptin and adiponectin levels between the obese subjects with asthma and obese subjects without asthma, and no significant differences in IL-6 and TNF- α levels were seen between the four study groups. The subjects with asthma differed significantly from the subjects without asthma in serum levels of ECP and total IgE and eNO levels. No significant differences were found in these variables between the obese subjects with asthma and normal-weight subjects with asthma, or between the obese subjects without asthma and healthy controls.

Associations between Leptin and Adiponectin Levels and Lung Functions, AHR to Methacholine, eNO, and Markers of Atopy

As shown in Table 3, FEF₂₅₋₇₅ levels were positively correlated with serum adiponectin levels ($r = 0.325$, $P = .048$). Neither serum leptin levels nor serum adiponectin levels were correlated with methacholine PC₂₀ levels in the subjects with asthma, and no significant correlations were found between serum leptin and adiponectin levels, eNO, and markers of atopy.

Association between Leptin and Adiponectin and Severity of EIB

As shown in Figures 2 and 3, after adjusting for BMI, serum leptin levels were positively correlated with maximum decreases in FEV₁ after exercise ($r = 0.432$, $P = .006$) and

Table 2. Biomarkers in the Peripheral Blood and eNO of the Study Subjects^b

	Subjects with asthma		Subjects without asthma		<i>P</i> ^a
	Obese ($n = 19$)	Normal weight ($n = 23$)	Obese ($n = 23$)	Normal weight ($n = 20$)	
Leptin (ng/mL)	14.14 $\pm$ 7.35 ^{c,e}	4.51 $\pm$ 2.61	12.64 $\pm$ 8.67 ^e	4.81 $\pm$ 3.64	<.0001
Adiponectin (μ g/mL)	5.10 $\pm$ 3.35 ^{c,e}	10.83 $\pm$ 5.42	7.28 $\pm$ 3.82 ^e	12.53 $\pm$ 5.02	<.0001
IL-6 (pg/mL)	5.26 (3.46–6.85)	5.26 (2.93–7.87)	5.09 (3.14–7.85)	4.71 (3.62–6.50)	.985
TNF- α (pg/mL)	6.36 (4.13–8.41)	7.29 (4.71–9.04)	6.98 (3.44–13.39)	6.48 (4.41–16.24)	0.953
Total IgE (IU/mL)	352.0 (150.3–547.9) ^{d,e}	219.0 (214.6–568.7) ^e	70.9 (34.4–79.6)	35.2 (12.6–62.4)	<.0001
PB eosinophil (/mL)	412.7 $\pm$ 50.5 ^{d,e}	383.9 $\pm$ 293.3 ^e	107.5 $\pm$ 18.9	143.8 $\pm$ 50.7	.005
ECP (ng/mL)	32.9 (16.3–52.7) ^{d,e}	29.1 (15.0–123.3) ^e	11.1 (4.8–21.1)	5.2 (5.1–12.3)	<.0001
eNO (ppb)	19.0 (14.75–35.0) ^{d,e}	19.0 (13.5–26.0) ^e	13.0 (10.0–19.0)	11.0 (8.0–13.0)	<.0001

Data are presented as mean $\pm$ standard deviation, or median (interquartile range).

^a Kruskal-Wallis test.

^b Post hoc pairwise comparisons are Tamhane tests.

^c $P < .05$ versus normal weight subjects with asthma.

^d $P < .05$ versus obese subjects without asthma.

^e $P < .05$ versus healthy.

Table 3. Partial Correlation Coefficients in the Study Subjects

	Serum leptin (ng/mL)		Serum adiponectin (μ g/mL)	
	<i>R</i> ^a	<i>P</i>	<i>R</i> ^a	<i>P</i>
FEV ₁ (pred %)	−0.021	.312	−0.100	.579
FVC (pred %)	−0.111	.537	0.066	.714
FEV ₁ /FVC ratio	−0.099	.584	0.330	.060
FEF _{25–75} (pred %)	−0.104	.564	0.325	.048
Methacholine PC ₂₀ (mg/mL) ^b	−0.031	.906	0.224	.377
Exhaled nitric oxide (ppb)	0.109	.548	−0.063	.826
Total IgE (IU/mL)	0.277	.119	−0.295	.244
PB eosinophil (/mL)	0.060	.742	−0.244	.171
ECP (ng/mL)	0.028	.878	−0.170	.106

^a Spearman correlation coefficients. Analysis is adjusted for BMI.

^b Methacholine PC₂₀ (mg/mL) are not applicable for children without asthma.

AUC₂₀ ($r = 0.486$, $P = .002$) in the subjects with asthma. After adjusting for BMI, serum adiponectin levels were negatively correlated with maximum decreases in FEV₁ after exercise ($r = -0.314$, $P = .046$) and AUC₂₀ ($r = -0.433$, $P = .024$). The odds for having EIB were incrementally higher for children with higher levels of serum leptin (Table 4). In the groups without asthma, neither serum leptin levels nor adiponectin levels were correlated with the maximum decrease in FEV₁ after exercise and AUC₂₀ after adjusting for BMI.

DISCUSSION

We have shown that levels of the adipocyte-derived hormones leptin and adiponectin were higher and lower, respectively, in obese subjects with asthma than in normal-weight subjects with asthma, and were significantly correlated with BHR induced by exercise challenge.

Some studies have concluded that obesity is a risk factor for BHR to methacholine,^{20,21} but others have reported no increase in BHR to methacholine with increasing BMI.^{22,23} The studies that used exercise challenge to examine BHR consistently showed that EIB was significantly higher in obese subjects with asthma than in nonobese subjects with asthma,^{24–27} and the current results agree with the findings of those studies. Exercise challenge causes airway smooth muscle contraction via one or more intermediate mechanisms, such as local and central neuronal reflexes and release of mediators such as leukotrienes, prostaglandins, and histamine by inflammatory cells, resulting in airway inflammation,²⁸ whereas methacholine acts directly on airway smooth muscle receptors, causing constriction independent of airway inflammation.

Exercise causes asthma attacks because of loss of water by evaporation from the surface of the airways when the inspired air is exposed to body conditions.²⁹ This loss of water increases airway osmolarity, which initiates activation of epithelial and mast cells and release of inflammatory mediators in the airways and thus bronchoconstriction.^{30–33} Mito et al³⁴ have reported increased mast cell numbers in the tracheal

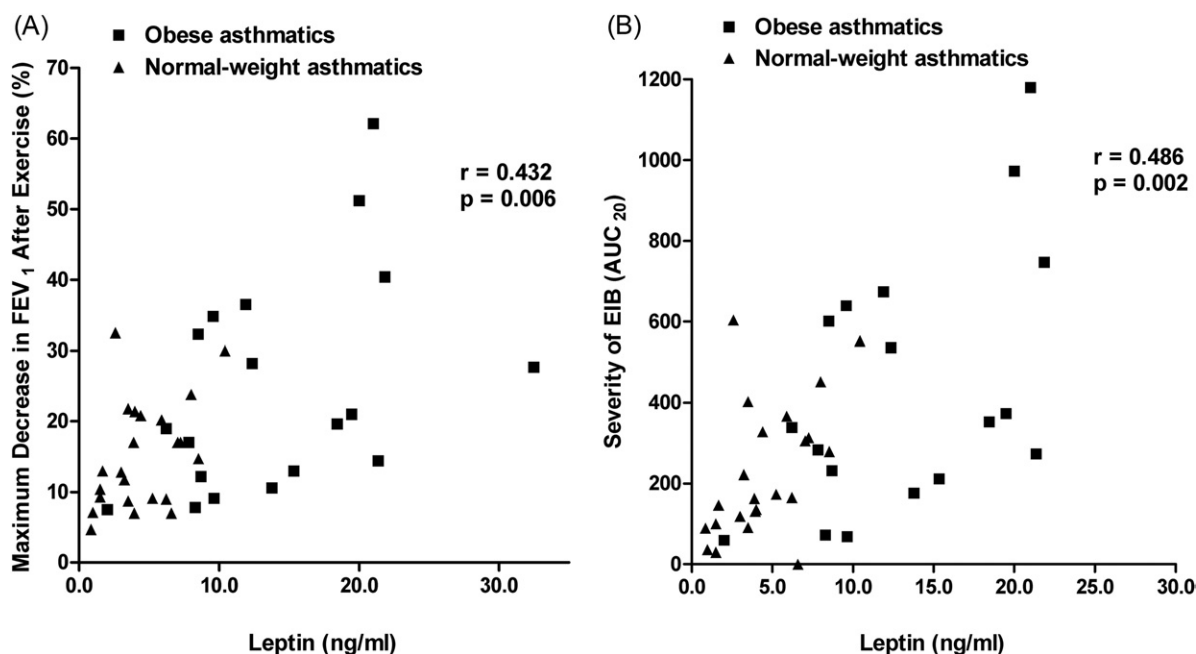


Figure 2. Relationships between serum leptin and maximum decrease in FEV₁ after exercise, and area under the FEV₁ curve over the first 20 minutes after exercise (AUC₂₀) in children with asthma. Serum leptin levels were significantly related to the A, postexercise maximum decrease in FEV₁ ($r = 0.432$, r = partial correlation coefficient adjusted for BMI, $P = .006$) and B, the severity of EIB (AUC₂₀) ($r = 0.486$, r = partial correlation coefficient adjusted for BMI, $P = .002$).

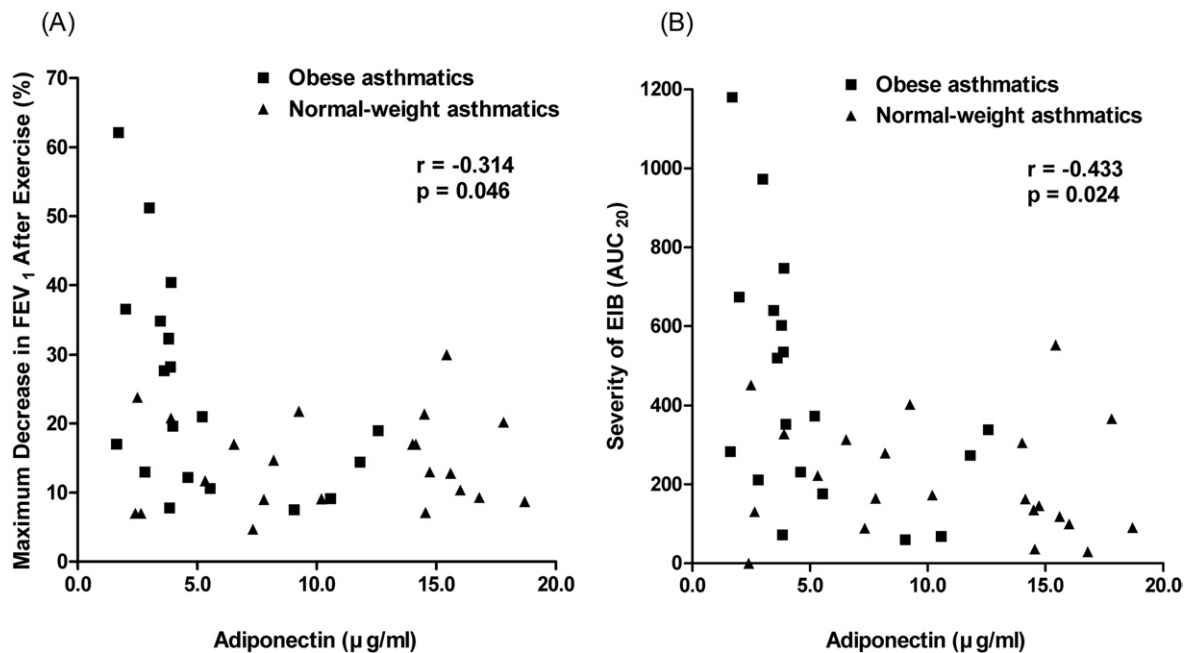


Figure 3. Relationships between serum adiponectin and maximum decrease in FEV₁ after exercise, and area under the FEV₁ curve over the first 20 minutes after exercise (AUC₂₀) in asthmatic children. Serum adiponectin levels were significantly related to the A, postexercise maximum decrease in FEV₁ ($r = -0.314$, r = partial correlation coefficient adjusted for BMI, $P = .046$) and B, the severity of EIB (AUC₂₀) ($r = -0.433$, r = partial correlation coefficient adjusted for BMI, $P = .024$).

mucosa of obese mice sensitized by OVA. Expression of 5-lipoxygenase activating protein is substantially increased in the adipose tissue of obese mice.³⁵ This could lead to the synthesis and release of large amounts of leukotrienes from activated mast cells and ultimately enhance airway responsiveness. Johnston et al¹⁰ have demonstrated that obese mice sensitized with ovalbumin have greater ovalbumin-induced increases in airway responsiveness than lean mice. These changes occurred in the absence of any effects on T helper cell 2 (Th2) cytokine production and despite reduced eosinophilic inflammation, suggesting that the modality of allergen-induced BHR in these obese mice is unlikely to involve augmented Th2-type inflammation. Instead, an increased role for mast cells or leukotrienes may exist in this situation.^{5,10} In this context, Peters-Golden et al³⁶ recently reported a more beneficial effect of the leukotriene antagonist montelukast in obese than lean individuals with asthma.

Shore et al⁹ observed that leptin treatment augmented allergen-induced BHR but did not affect eosinophil influx or

Th2 cytokine expression in a mouse model, suggesting that leptin is capable of augmenting BHR through a mechanism independent of Th2 inflammation. They also showed in mice that administration of adiponectin resulted in almost complete suppression of allergen-induced BHR, airway inflammation, and Th2 cytokine expression in the lung.³⁷ Taideman et al³⁸ recently demonstrated expression of leptin receptors in mast cells of human lung tissue, which suggests that leptin has paracrine or autocrine immunomodulatory effects on mast cells. Mast cell activation is a feature of EIB.^{30–33} This supports the idea that the high leptin levels in the obese subjects with asthma during exercise challenge were responsible for increasing mast cell activation and BHR in the current study, although further work is necessary to clarify this.

Postexercise FEV₁ decreased markedly in both the obese subjects with asthma and normal-weight subjects with asthma, although the obese subjects without asthma, who had higher leptin and lower adiponectin levels, did not show significant exercise-induced BHR. Because BHR to exogenous stimuli is a characteristic feature of asthma³⁹ and we excluded individuals with positive methacholine challenge for BHR from the groups without asthma, the obese subjects without asthma might not show EIB. These findings suggest that leptin levels are associated with exercise-induced BHR in a synergistic manner in asthmatic subjects but not in subjects that are only obese. Leptin levels might play a role in augmenting exercise-induced BHR in subjects with asthma, as demonstrated in the Shore's study in mice⁹.

Table 4. Prediction of Exercise-Induced Bronchoconstriction

	Odds ratio	95% CI	P
Sex	1.3	0.434–4.137	.612
Age	0.6	0.414–0.967	.115
BMI	0.9	0.687–1.138	.340
Leptin	1.2	1.027–1.335	.019
Adiponectin	0.9	0.789–1.035	.142

Analysis is adjusted for age, sex, BMI, and adipokines.

Data on pulmonary function in obese adults have been well reported, whereas data from similar studies in the pediatric population are limited and conflicting. We documented lower baseline values of FEV₁, FEV₁/FVC, and FEF₂₅₋₇₅ in the obese subjects with asthma and obese subjects without asthma than in the healthy controls. Similar data were obtained by Spathopoulos et al,⁴⁰ who demonstrated in a large cohort of prepubertal children with obesity or asthma that pulmonary function was characterized by reductions in FEV₁, FVC, FEF₂₅₋₇₅, and FEV₁/FVC. We found no further significant differences in FEV₁, FVC, or FEV₁/FVC ratio between the obese subjects with asthma and normal-weight subjects with asthma, except for FEF₂₅₋₇₅. This outcome is similar to that of a very recent study that reported no relationship between BMI in subjects with asthma and spirometry findings in a large prospective disease management trial.⁴¹ Only asthma patients were included in that study. In our study, no significant differences were found in bronchodilator responses between the obese subjects without asthma and healthy controls. In agreement with our data, Bibi et al⁴² demonstrated in a large population of prepubertal children that bronchodilation could not be induced by salbutamol inhalation in obese subjects without asthma. No significant differences were seen in bronchodilator responses between the obese subjects with asthma and normal-weight subjects with asthma in the current study.

Tumor necrosis factor alpha and IL-6 are the two best-studied cytokines associated with obesity, and they have consistently been found to be elevated in the sera or adipose tissue, or both, of obese subjects.⁴³ However, we found no significant differences in IL-6 and TNF- α levels between the four study groups. Exhaled nitric oxide has been the noninvasive biomarker most widely used for evaluating airway inflammation in asthma.^{44,45} In our study, eNO levels were elevated in the subjects with asthma, but we observed no difference in eNO level in obese versus normal-weight asthmatics and no significant correlations between serum leptin and adiponectin levels and eNO.

Many unresolved questions persist about the mechanisms involved in EIB in asthmatic airways. One limitation of our study was the small sample size. Nevertheless, our study design had strengths. As far as we are aware, this is the first controlled observational study designed to explore the relationship between the adipokines and BHR with exercise. We found that levels of serum leptin and adiponectin were significantly correlated with BHR induced by exercise challenge. The finding of the study of Shore in mice⁹ that leptin treatment augmented allergen-induced BHR raises the possibility that the changes in leptin and adiponectin levels are causative of EIB/BHR, although we did not ourselves obtain any direct evidence for a cause-and-effect relationship. Further studies are warranted to see whether leptin and adiponectin augment and inhibit, respectively, bronchial epithelial cell activation, mast cell activation, and inflammatory mediators release during exercise-induced bronchoconstriction in asthmatics. If such a relationship is demonstrated, treating EIB/

BHR with agents inhibiting leptin and others enhancing adiponectin may be possible.

ACKNOWLEDGMENTS

We thank our pediatric patients and their parents for generous donation of study samples, Mr. Soon-H.O. Koo for technical assistance, and Professor Yeong-Gwan Im and Mr. Han-Sang Baek for data management. We also thank Aerocrine for their support of NIOX MINO for eNO measurement.

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