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# Relationship between the Serum Leptin Concentration and Bronchial Hyperresponsiveness in Preschool Children

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## ABSTRACT

**The objective of this article was to investigate the relationship between serum leptin concentration and airway inflammation in preschool children with asthma, the individual serum leptin concentration, obesity index, and respiratory function, including bronchial hyperresponsiveness (BHR), were investigated. The relationship between the serum leptin concentration and the obesity index was determined in 103 healthy preschool children aged 6 as controls. For the BHR investigation, the serum leptin concentration in 57 preschool children aged 6 (33 with asthma and 24 wheezers) was determined. These children then underwent lung function tests using methacholine inhalation challenge by the oscillation method. The BHR was also measured in 15 of the 103 controls subjects. In the control group, serum leptin concentration was significantly correlated to the obesity index. There was no statistical difference in both serum leptin concentration and the obesity index among the asthma patients, the wheezers and the control group. Although there was no significant association between serum leptin concentration and lung function parameters (FVC, $FEV_1$, and V′50), a significant association was observed between the serum leptin concentration and BHR ($p < 0.038$), and between obesity index and BHR ($p = 0.015$). The serum IL-6 concentration was not related with the serum leptin concentration and BHR. These results suggest that serum leptin has no effect on lung functional parameters but does affect the BHR, and that the relationship between leptin and airway inflammation may contribute to the severity of childhood asthma at 6 years of age. (Pediatr Asthma Allergy Immunol 2008; XX[X]; 000–000.)**

## INTRODUCTION

A VARIETY OF INFLAMMATORY MARKERS such as TNF-$\alpha$, IL-6, and C-reactive protein are increased in obese compared to thin subjects.[1–3] Recent studies have led to the conclusion that adipose tissue is no longer just a simple storage depot for body energy but is an active participant in the regulation of physiologic and pathologic processes, including inflammation.[4]

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Obesity has been shown to be associated with several types of inflammatory-autoimmune disorders, including inflammatory bowel disease, rheumatoid arthritis, and multiple sclerosis.[5–7] Therefore obesity may be directly involved in the formation of tissue inflammation. Asthma is known to develop in response to chronic airways inflammation. An association between asthma and obesity was first reported in 1986.[8] This led to, a surge of interest in this relationship, thus resulting in the completion of over 30 cross-sectional and case–control studies on this topic over the course of the next 10 years.[9,10]

Indeed, adipose tissue produces and releases a variety of pro-inflammatory and anti-inflammatory factors, including leptin.[1] Leptin plays an important role in the regulation of body weight,[11] is an important research tool for investigating the regulation of body weight.[12] Animal experiments observing the effects of leptin and obesity on airway inflammation in response to both allergic and nonallergic exposures have suggested that an airway inflammatory response is enhanced by both endogenous and exogenous leptin.[13] Even in humans, there are some studies suggesting that leptin contributes to severity of ashma observed in obese subjects, especially children.[14,15]

However, few studies have observed the relationship between the severity of asthma and the concentration of leptin in serum,[16] and none have examined respiratory function tests such as bronchial hyperresponsiveness (BHR) in preschool children. BHR is the most important physiological aspect in asthma, and the association between airway inflammation and BHR has been well documented.[17,18] Some reports have suggested that the concentration of BHR is more closely related to severity of asthma than lung function parameters.[19,20] BHR is observed even in infantile asthma patients,[21] and persists after the diagnosis of asthma.[22] However, in younger children, the effect of serum leptin on the exacerbation of asthma and/or BHR is still unclear. To answer this question, serum leptin concentration was compared to the obesity index and lung function tests (including BHR) and the serum IL-6 level in 6-year-old preschool children during checkups performed in a village of Gunma prefecture in Japan between April 2001 and March 2006.

## MATERIALS AND METHODS

### *Study protocols*

In healthy 6-year-old preschool children, serum leptin concentrations and obesity index were measured for control data. In 6-year-old preschool children with asthma and wheezing, serum leptin concentration and obesity index were measured, and lung function tests were administered, including methacholine inhalation challenge using the oscillation method described.[23,24]

### *Subjects*

The serum leptin concentration and the obesity index were measured in 103 healthy preschool children aged 6 (boys:girls = 58:45) for control data. The obesity index = weight/standard weight where standard weight = $(\text{height})^2 \times 22$. Fifty-seven preschool children; 33 asthma (boys:girls = 22:11), and 24 wheezers (boys:girls = 9:15) were enrolled in the study (Table 1). In addition, 15 of the 103 controls (boys:girls = 9:6) agreed to participate in the BHR measurement (Table 1). The weight and height in all subjects were measured in the public health center. The clinical diagnosis of bronchial asthma was based on a characteristic history of recurrent attacks of dyspnea with perceptible wheezing.[25] All asthma subjects were diagnosed by physicians, and were experiencing atopic asthma due to their reaction to common environmental allergens administrated to the skin and the results of radioallergosorbent tests (development of a wheal 5 mm or larger in a prick test and more than 0.70 PRU in the RAST was defined as 2+ of reactivity, respectively). Prior to the methacholine inhalation challenge, no child was undergoing long-term steroid inhalation therapy.

The wheezers were diagnosed according to their history of wheezing without dyspnea, and wheezing was not detected more than three times over the life time. Based upon a questionnaire from the public health office for preschool children, the age-matched controls were free of chronic respiratory diseases.

All of the children had been free of respiratory tract infections for more than 4 weeks prior to enrollment, and had received no medication for at least 12 hours prior to the testing. Approval was obtained from the hospital ethics committee, and informed parental consent for each child was obtained before starting the study.

TABLE 1. PROFILES OF THE SUBJECTS WHO WERE 6 YEARS OF AGE

| | n | *Sex (Boy:Girl)* | *FVC (% pred)* | *$FEV_1$ (% pred)* | *OI%* |
|---|---|---|---|---|---|
| Control 1 | 103 | 58:45 | N.D. | N.D. | 5.1 ± 14.1 |
| Control 2[a] | 15 | 9:6 | 88.4 ± 10.2 | 90.4 ± 11.6 | 4.8 ± 8.6 |
| Asthma | 33 | 22:11 | 85.8 ± 13.0 | 95.5 ± 28.4 | 3.3 ± 16.1 |
| Wheezers | 24 | 9:14 | 91.3 ± 23.6 | 93.1 ± 19.9 | 3.0 ± 11.7 |

Data show mean ± SD. N.D., not done; OI%, obesity index %; [a]Control 2 were children who agreed to undertake the methacholine inhalation challenge in control group 1.

### *Methacholine inhalation challenge*

Methacholine inhalation challenge was performed on all 72 children, as previously described.[26] The children underwent the challenge in a seated position wearing a nose clip. An Astograph (TCK 6100H, Chest Co., Tokyo, Japan) was used as the inhalation device and the monitor of real-time respiratory resistance (Rrs) (Fig. 1).

A constant amplitude pressure generator was connected to the mouthpiece and produced a constant amplitude pressure of sine waves at the mouth using a loudspeaker box system. A constant bias flow was introduced between the nebulizer and the flow meter to minimize the dead space. The airflow at the mouth was measured with a pneumotachometer, and a differential pressure transducer was also used to measure the mouth pressure relative to the ambient pressure. To record the dose–response curves, the respiratory resistance (Rrs) was calculated using an analog computer and then was continuously displayed on the time scale of an X-Y recorder.

The methacholine inhalation challenge was performed according to the procedure described by Takishima.[27] Briefly, the methacholine (Daiichi Kagaku Yakuhin Co., Tokyo, Japan), prepared on the day of the test, was serially diluted twofold with saline (10 dose steps from 25 mg/mL to approximately 49 $\mu$g/mL) and administered via an Astograph. The system consisted of 12 identical nebulizers connected to a main tube and an air compressor that switched from one nebulizer to the next at 1-minute intervals. Saline was

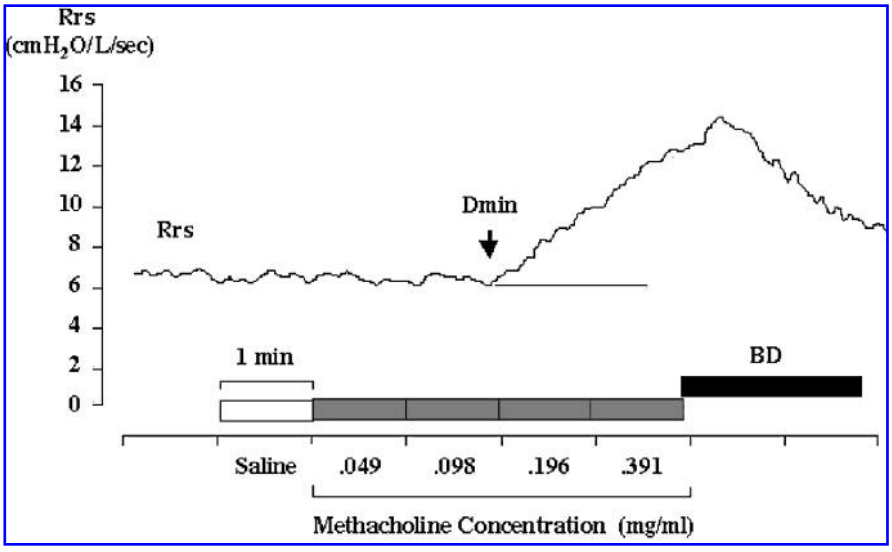


**FIG. 1.** Methacholine inhalation challenge using the oscillation method. Dmin is the cumulative dose of methacholine at the inflection point at which Rrs increased linearly, and was recorded as the BHR.

set up in the first nebulizer as the control. The 12th nebulizer contained salbutamol hemisulfate (1.7 mg/mL in saline), and it was administered for 2 minutes.

Subsequent doses of methacholine were then doubled until a 200% increase in Rrs from the baseline was reached. Dmin is the cumulative dose of methacholine at the inflection point at which Rrs increased linearly, and was recorded as the BHR (Fig. 1). One Dmin unit was considered to be equal to 1 minute of inhaling an aerosolized methacholine solution (1.0 mg/mL) during tidal breathing. Routine auscultation was performed before and during the methacholine challenges to document wheezing.

### *Measurement of leptin and IL-6*

The serum concentrations of leptin and IL-6, which are related to obesity, were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Quantikine; R&D Systems, Minneapolis, MN) in accordance with the manufacturer's instructions. The assays could detect leptin or IL-6 concentrations above a baseline of 3 pg/mL.

### *Data analysis*

All analyses were carried out using an SPSS statistical software package, version 13.0J (SPSS Japan, Tokyo). The normality of the distribution was assessed through the use of the Kolmogorov-Smirnov test. A parametric analysis of variance (ANOVA) was performed to determine the significant variance between the groups. Correlations between Dmin and other results were assessed with the Spearman rank correlation coefficient. The relationship between the serum leptin concentrations and Dmin after adjusting for the obesity index by a partial correlation analysis was examined. The data are expressed as the means ± SD. For all analyses a two-sided probability value below 0.05 was considered to indicate statistical significance.

## RESULTS

### *Serum leptin concentration and obesity in the control group*

The serum leptin concentration in 103 healthy 6-year-old Japanese children was measured by ELISA. There was a significant relationship between the serum leptin concentration and the obesity index ($p <$

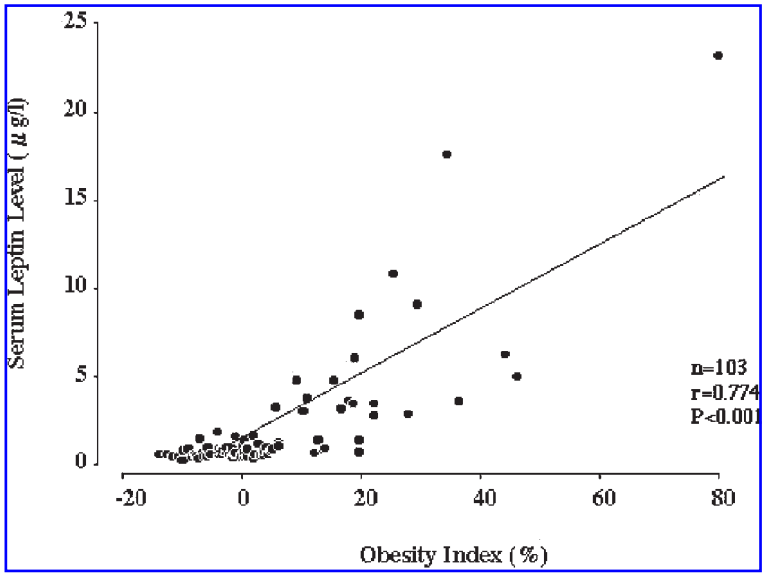


**FIG. 2.** Relationship between the obesity index (%) and leptin in controls, aged 6. The serum leptin concentration in 103 healthy 6-year-old Japanese children was measured. There was a significant relationship between the serum leptin concentration and the obesity index.

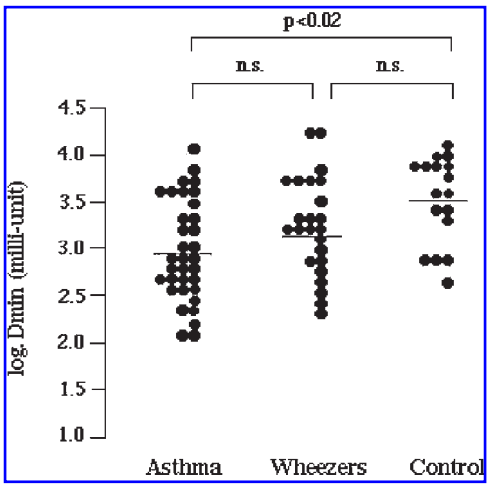


**FIG. 3.** Comparison of Dmin among patients with asthma, wheezers, and control patients. There was a significant difference in Dmin between the asthma and the control groups. Asthma, $n = 34$; wheezers, $n = 24$; controls, $n = 17$.

0.001, Fig. 2). In addition, the serum leptin concentration and the obesity index in the asthma and other groups ($n = 72$) were calculated. There was a significant relationship between them ($p = 0.0363$, data not shown).

### *Serum leptin concentration and lung function*

The mean baseline forced expiratory volume in 1 second ($FEV_1$) was 95.5% in the asthma group, 93.1% in the wheezers group, and 90.4% in the control group. Baseline $FEV_1$ exceeded 70% of the values predicted in all participants. The methacholine inhalation challenge was then administered. There was a significant difference in Dmin between the asthma and the control groups (Fig. 3). Consistent with previous

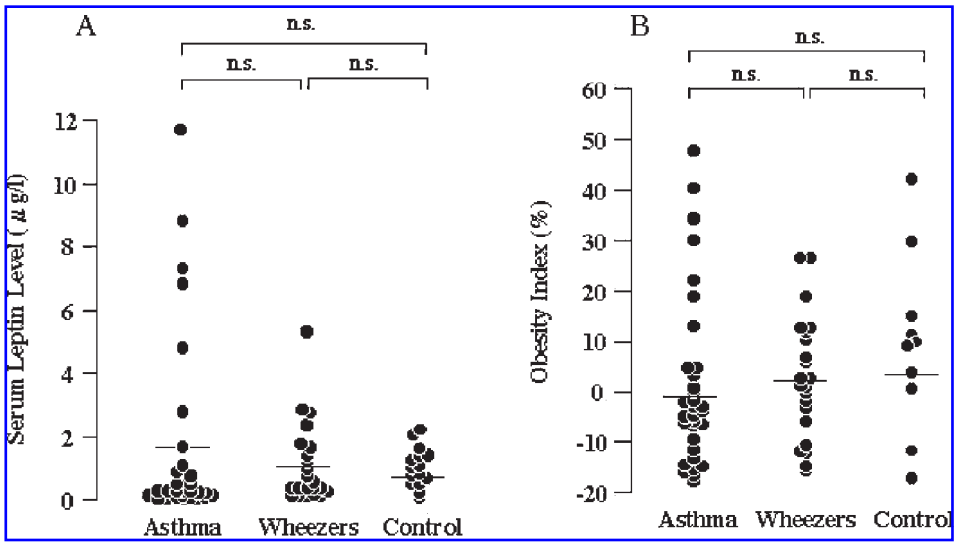


**FIG. 4.** Comparison of serum leptin concentration and the obesity index (%). There was no statistical difference observed in the serum leptin concentration among three groups, but the mean serum leptin concentration in the asthma group was higher than that in the control group (**A**). Also, there was no difference in obesity index among the three groups (**B**). Asthma, $n = 34$; wheezers, $n = 24$; controls, $n = 17$.

TABLE 2. RELATIONSHIP AMONG THE LUNG FUNCTION, THE SERUM LEPTIN LEVEL, AND THE OBESITY INDEX (%)

| | *Serum leptin level* | *OI%* |
|---|---|---|
| FVC (pred%) | 0.024 ($p = 0.852$) | 0.072 ($p = 0.564$) |
| $FEV_1$ (pred%) | 0.016 ($p = 0.898$) | 0.040 ($p = 0.691$) |
| $V\cdot_{50}$ (pred%) | 0.114 ($p = 0.377$) | 0.040 ($p = 0.743$) |
| log.Dmin (milli-unit) | 0.244 ($p = 0.038$) | 0.274 ($p = 0.015$) |

OI%, obesity index (%).

studies, the Dmin in the asthma group was the lowest, thus indicating that BHR was the highest among the three groups. Dmin of wheezers was halfway between the asthma and control groups.

Comparison of the serum leptin concentrations in the three group is shown Figure 4A. The serum leptin concentration in the asthma group was higher than that in the control group. However, the difference was not statistically significant. In addition, comparison of the obesity index is shown Figure 4B, and there was no difference in obesity index among the three groups.

The results of the relationship between the lung function tests, including BHR measurement, the serum leptin concentration and the obesity index are shown in Table 2. Although there was no relationship between the parameters of other lung function test and the serum leptin concentration, Dmin showed a significant correlation with the serum leptin concentration ($p = 0.0038$; Table 2 and Fig. 5). Dmin also showed a correlatation with the obesity index ($p = 0.015$, respectively; Table 2 and Fig. 6). The correlation was confirmed by the the nonparametoric method, the Spearman rank correlation coefficient, between the Dmin and the serum leptin concentration ($p = 0.0432$), and between Dmin and the obesity index ($p = 0.0364$).

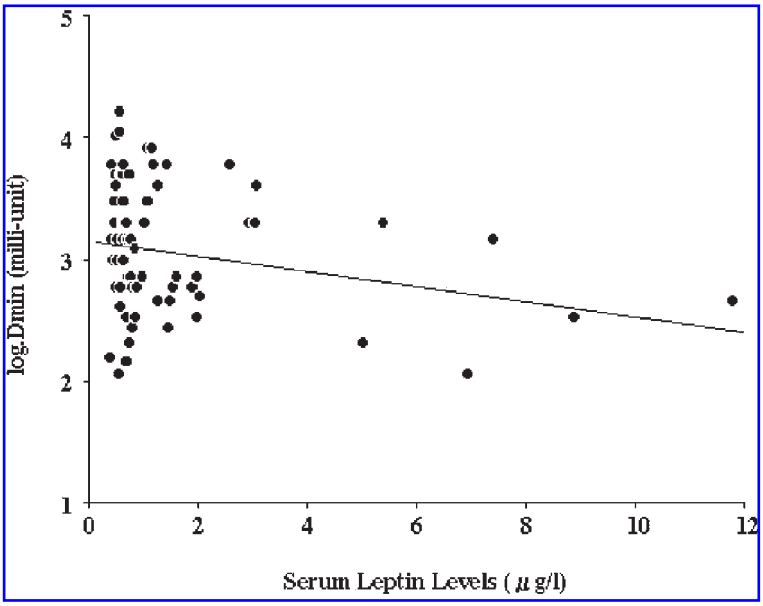


**FIG. 5.** Relationship between Dmin and serum leptin concentration. Although there was no relationship between the parameters of other lung function test and the serum leptin concentration, Dmin showed a significant correlation with the serum leptin concentration.

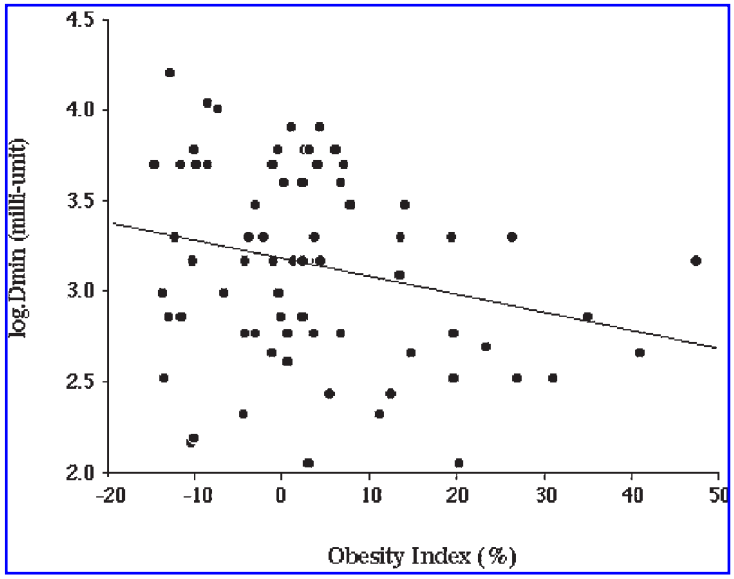


**FIG. 6.** Relationship between Dmin and the obesity index (%). Dmin showed a correlatation with the obesity index.

However, no apparent relationship between serum leptin concentrations and Dmin after adjustment for the obesity index by partial correlation analysis was observed ($p > 0.1$; data not shown).

### *Serum leptin concentration and serum IL-6 concentration*

The serum IL-6 concentration was also investigated in the three groups, but there was no relationship between IL-6 concentration and the serum leptin concentration, the rate of deviation from mean leptin concentration and the obesity index. In addition, no relationship was observed between serum IL-6 concentration and each parameter of lung function tests including the BHR measurement (data not shown).

## DISCUSSION

Many physiological studies have suggested that obesity causes a reduction in respiratory compliance, lung volumes, and peripheral airway diameter.[28–31] Therefore, an obesity was suspected to have an effect upon BHR. A study of European Community Respiratory Health Survey demonstrated that BHR increases with the body mass index (BMI) in men but not women.[32] In pediatrics, it was also suggested that BMI had an association with BHR in school-age boys,[33] and that BMI is a significant indicator of atopy, allergic symptoms, and BHR in teenage girls.[34]

However, there is no relationship between the lung function and BHR;[24] that is, the anatomical construction of the lung and the physiological function in the respiratory system are not identical. BHR is mainly affected by airway inflammation, and is among the best indicators for the presence of airway inflammation.[35] Indeed, some reports have suggested contrasting results, namely, that the BMI is associated with the diagnosis of asthma and the symptoms of dyspnea and wheezing, but is not associated with BHR in adults[36] and children.[37] Therefore, more clinical examination on the relationship between obesity and BHR are required.

Initially, the serum leptin concentration in healthy 6-year-old Japanese children was measured by ELISA, and this indicated statistically, that serum leptin concentration was related to the obesity index in the control children ($n = 103$, $p < 0.001$). This was also observed in the children with asthma and wheezing ($n = 72$, $p = 0.0363$). In this type of study, it is very important to use same-aged controls, because the serum leptin concentration is strongly affected by aging,[38] and clinical effect of leptin may be different in each

age group. In addition, BHR was strongly affected by age,[23] and clinical effect of BHR is different at each age.[39] These data suggest that obesity may potentially influence the serum leptin concentration in children aged 6.

Second, the relationship between the serum leptin concentration and the lung function including BHR was evaluated. It is very interesting that there was no significant association between plasma leptin concentration and representative functional parameters in the lung, including FVC, $FEV_1$, and $V'50$, whereas an apparent association between serum leptin concentration and Dmin was found. The difference in the serum leptin concentration and the obesity index was observed among three groups (those with asthma, the wheezers, and the control groups), and there was no significant difference among them. These results suggest that the serum leptin level therefore has some effect on BHR development regardless of the clinical aspects.

The effect of leptin on lung physiology is poorly understood. However, some previous data suggests that leptin concentrations increase during inflammation, in turn, leptin may promote airway inflammation. Exogenous leptin will modulate allergic airway responses in mice. Furthermore, inhaled ovalbumin challenge in leptin-treated mice is associated with both BHR enhancement and serum IgE.[13] In clinical reports, Guler et al.[16] studied the association of leptin concentration and asthma in children with a mean age of 6 years, and demonstrated a significant relationship was detected in boys. Considering these results, it is possible that leptin may therefore induce an upregulation of inflammatory cytokines, thus causing airway inflammation and eventually resulting in increasing BHR in preschool children.

However, no apparent correlation between the serum leptin concentration and Dmin value adjusted by the obesity index was observed based on a partial correlation analysis. Previous studies reported that the BMI is associated with existing asthma in women, and this association is not significantly associated with serum leptin concentration.[15] Furthermore, Shore et al.[40,41] reported that ozone exposure caused a significant increase in pulmonary resistance, and increased airway responsiveness in fasting mice accompanied with a marked reduction in serum leptin concentration. If the effect of serum leptin on airway inflammation was biphasic, the adjustment of Dmin by obesity index would not demonstrate a simple correlation between serum leptin concentration and Dmin value. This indicates that, in preschool children, the effect of serum leptin on BHR may be complex, and that leptin-activated multi-inflammatory pathways may totally account for BHR development. More testing in larger sized patient groups is thus required to clarify the relationship between serum leptin concentration and Dmin value.

The expression of the adipokines including TNF-$\alpha$, IL-1, IL-6, MCP-1, eotaxin, IL-8, and MIP-1 may possibly increase with obesity and exacerbate airway inflammation or airway obstruction in asthma.[42] Some *in vitro* studies suggest that leptin directory induces IL-6 transcription and secretion in some cell types.[40,43] However, this clinical investigation of serum IL-6 concentration in the same subjects showed no significant relationship between serum IL-6 concentration, serum leptin concentration, obesity index, and increased BHR. In contrast, inflammatory cytokines such as TNF-$\alpha$ and IL-1$\beta$ have been shown to induce the release of leptin from adipocytes, and it is conceivable that airway inflammation in asthmatics induces an increased leptin concentration. It is impossible to ignore the direct correlation between the serum leptin expression and airway inflammation which induces BHR. Further investigations are therefore needed to resolve this question.

In conclusion, these results suggest the possibility that high serum leptin concentration affect the BHR, while also contributing to the severity of asthma in children aged 6. This is the first study to examine the relationship between obesity and BHR using methacholine inhalation challenge in age-limited preschool children. However, our findings did not show any direct evidence that obesity enhances airway inflammation which thus induces BHR. Further investigations are therefore needed to understand the effect of obesity on BHR in preschool children.

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## DISCLOSURE STATEMENT

The authors declare there are no competing financial interests.

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