# Impact of Serum Adiponectin, Plasminogen Activator Inhibitor-1, and Oxidative Stress Levels on Eosinophilic Inflammation of the Airway and the Whole Body in Children with Obesity

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# Impact of Serum Adiponectin, Plasminogen Activator Inhibitor-1, and Oxidative Stress Levels on Eosinophilic Inflammation of the Airway and the Whole Body in Children with Obesity

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**Background:** Childhood obesity has been suggested as a risk factor for bronchial asthma. The aim of this study was to clarify the relationship between clinical factors related to obesity, including adipocytokines and eosinophilic inflammation, as candidate pathogenesis mechanisms of bronchial asthma in Japanese children and adolescents.

**Methods:** Forty-one children and adolescents visiting our outpatient clinic were enrolled. The relationship between participants' clinical and demographic characteristics and fraction of exhaled nitric oxide (FENO) or blood eosinophil (B-Eos) count values were analyzed.

**Results:** No significant correlation was observed between either FENO or B-Eos count values and the body mass index z-score. FENO was high (≥35 ppb) in 8 cases and normal (<35 ppb) in 33 cases. Adiponectin was significantly lower in the high FENO group than in the normal FENO group (6.5 vs. 8.1 μg/mL, $p<0.02$). A negative correlation between B-Eos count values and adiponectin levels ($r=-0.34$, $p<0.05$) and a positive correlation between B-Eos count values and plasminogen activator inhibitor-1 levels were noted ($r=0.42$, $p<0.01$). The univariable odds ratio of adiponectin for high FENO was 0.62 (0.41-0.95) and the association was borderline after adjusting for B-Eos.

**Conclusions:** Eosinophilic inflammation was associated with a decrease in serum adiponectin levels which may be induced by visceral fat accumulation.

**Key Words**: adiponectin, childhood obesity, eosinophilic inflammation, bronchial asthma, fraction of exhaled nitric oxide

## Introduction

Obesity is well known as a major risk factor for the development of bronchial asthma.[1-3] However, the mechanisms involved in this relationship remain unclear. Inflammatory changes within adipose tissue of adult women with obesity could contribute to airway inflammation and airway reactivity in individuals with obesity.[4]

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In later childhood, childhood bronchial asthma is mainly caused by eosinophilic inflammation of the airway. However, the pathological association between body fat accumulation and eosinophilic inflammation of the airway is unclear, in particular, in children.

Boulet asserted that low-grade systemic inflammation caused by adipocytokines and oxidative stress is a risk factor for bronchial asthma.[5] In adipocytokines, adiponectin and leptin play an important role in low-grade inflammation. Adiponectin, an insulin-sensitizing hormone, has predominantly anti-inflammatory and antioxidative effects.[6] Adiponectin has receptors in the respiratory epithelium and smooth airway muscles.[7,8] Adiponectin levels are decreased in Japanese children with obesity who have high visceral fat accumulation.[9]

Leptin, an interleukin (IL)-6-like adipocytokine secreted from adipocytes, promotes inflammation and oxidative stress. Leptin receptors are present in the respiratory epithelium, submucosal space, and activated lymphocytes in the lung.[10,11] Leptin levels increase in proportion to subcutaneous fat accumulation.[12] In fact, among middle-aged women, an increase in leptin levels has been related to bronchial asthma.[13]

Plasminogen activator inhibitor-1 (PAI-1), an adipocytokine, plays an important role in a range of physiological and pathological processes, including fibrinolysis, coagulation, inflammation, and wound healing. PAI-1 levels are elevated in insulin-resistant subjects and are associated with an increased cardiovascular risk of atherothrombosis.[14,15] Several studies have suggested that visceral adipose tissue is the major component affecting the relationship between elevated circulating PAI-1 and insulin-resistance syndrome.[15,16] We have previously demonstrated that the plasma PAI-1 levels of Japanese children and adolescents with obesity were significantly correlated with immunoreactive insulin, homeostasis model assessment insulin resistance, and quantitative insulin sensitivity check index values.[17]

Obesity is heterogeneous in children; its characteristics depend on the distribution of visceral and subcutaneous fat accumulation. The heterogeneity of simple obesity in terms of insulin resistance in Japanese children and adolescents has been previously demonstrated.[18] In the present study, we hypothesized that eosinophilic inflammation of the airway in children with obesity may be differ between visceral fat-dominant obesity or subcutaneous fat-dominant types of obesity. If eosinophilic inflammation of the airway is largely influenced by visceral fat, this type of inflammation might be associated with the presence of adiponectin and PAI-1. In contrast, if eosinophilic inflammation of the airway is influenced mainly by subcutaneous fat, this type of inflammation might be associated with the presence of leptin.

Malinovschi et al. have reported fraction of exhaled nitric oxide (FENO) and blood eosinophil (B-Eos) counts as independent risk factors for bronchial asthma.[19] FENO is mainly triggered by IL-4 and IL-13 and indicates eosinophilic inflammation of the airway. B-Eos is mainly triggered by IL-5 and indicates eosinophilic inflammation of the whole body.[19-21]

In the present study, we investigated the association between adiponectin, leptin, PAI-1, and oxidative stress levels, and eosinophilic inflammation of the airway in children with obesity by measuring FENO and B-Eos counts.

## Materials and Methods

### 1. Subjects and study design

We conducted a cross-sectional observational study with Japanese children and adolescents aged 6-15 years. Subjects were recruited from among patients who attended our outpatient clinic (Tokyo Women's Medical University Medical Center East, Tokyo, Japan) for treatment of obesity or an allergy-related disease (bronchial asthma, atopic dermatitis, food allergy) between September 2013 and August 2016. No limitations were placed on the weight of potential participants; however, participants with moderate or severe obesity were expected to be included. The exclusion criteria were: (1) use of inhaled or nasal corticosteroids in the 4 weeks before study participation, as corticosteroids reduce inflammation; (2) presence of endocrine metabolic diseases (e.g., diabetes mellitus), as these diseases can induce systematic inflammation; and (3) infection within 1 month before recruitment, as it would preclude distinguishing between infection and obesity as the cause of systematic inflammation. All data were collected 1-2 weeks after participant recruitment.

### 2. Tests and data collection

Medical history regarding bronchial asthma, allergic rhinitis, atopic dermatitis, and food allergies was obtained from the participants' parents. All participants visited our outpatient clinic in the morning after an overnight fast. Body height, weight, waist circumference, blood pressure, and body fat percentage were measured; 14 mL blood samples were also collected. Participants were required to remove their shoes and outer clothes for the measurements of body height and weight. Participants were asked to stand, take off their clothes, and breathe out slightly during the measurement of waist circumference, which was collected at the level of the umbilicus. Body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters ($kg/m^2$). The reference used for the calculation of the standard deviation score for BMI was based on the data from Japanese children published in 2000.[22] For blood pressure measurements, participants were required to sit in a relaxed state. Body fat percentage was determined using dual-energy X-ray absorptiometry (Lunar iDXA, GE Healthcare, Japan).

FENO was measured using the NIOX MINO® (Aerocrine, Solna, Sweden), an electrochemical sensor device, according to the guidelines of the American Thoracic Society (ATS) Committee on interpretation of FENO levels for clinical applications.[23] Participants were required to exhale for 10 seconds at an exhalation pressure of 10-20 $cmH_2O$ to maintain a stable flow rate of $50 \pm 5$ mL/s, which is the standardized measurement for lower respiratory tract FENO agreed upon by the European Respiratory Society and ATS.[24] A calibrated electrochemical sensor was used to evaluate the final 3 seconds of the exhalation; the results were expressed in parts per billion (ppb) with a range between 5 and 300 ppb.[25]

In the blood examination, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GTP), triglycerides (TGs), total cholesterol, high-density lipoprotein (HDL) cholesterol, uric acid, and blood glucose levels were measured using an automated analyzer (Labospect008, Hitachi High-Technologies, Tokyo, Japan). Immunoreactive insulin level was measured using a chemiluminescent enzyme immunoassay (Lumipulse Presto II, Fujirebio Inc., Tokyo, Japan). Low-density lipoprotein (LDL) cholesterol level was calculated using the Friedewald equation. Leptin (double-antibody radioimmunoassay method using a γ-counter), adiponectin (latex immunoassay turbidimetric method [Biomajesty JCA BM8000 series, Japan Electronics Co., Ltd, Tokyo, Japan]), and PAI-1 (latex coagulation method [Biomajesty JCA-BM9130, Japan Electronics Co., Ltd, Tokyo, Japan]) levels were also measured. Malondialdehyde-modified low-density lipoprotein (MDA-LDL) cholesterol level was measured as an oxidative stress marker. B-Eos values were measured using an automated analyzer (XN-3000, Sysmex, Okayama, Japan). The total immunoglobulin E (IgE) level was measured using a fluorescence enzyme immunoassay (Phiadia5000, Thermo Fisher Diagnostics, Massachusetts, USA), and high-sensitivity C-reactive protein level was measured using nephelometry (BN II, Siemens Healthcare Diagnostics, New York, USA).

### 3. Statistical analysis

Participants were divided into two groups (FENO-low: <35 ppb and FENO-high: ≥35 ppb), following the ATS clinical guidelines on FENO measurements.[23] We compared the baseline characteristic of these groups using the Wilcoxon rank sum test and Fisher's exact test. Pearson's correlation coefficient was used to calculate univariable correlation coefficients for FENO and B-Eos, and other variables. In addition, we fitted a logistic regression model for high-FENO (≥35 ppb) group. The variables of p value <0.1 in the single correlation coefficient with FENO were put on the calculation. We selected the variables for the multivariable logistic regression model for the high-FENO group in a stepwise selection process, whereby the factors that met Akaike's minimum information criterion (AIC) were considered the best match. Then, multivariable logistic regression model was used to calculate odds ratios. Two-tailed p values <0.05 were considered statically significant. JMP Pro 15 (SAS Institute, Cary, NC, USA) was used for statistical analyses.

### 4. Ethics

Written informed consent to participate in this study was obtained from patients' parents, and assents were provided. This study was conducted with the approval of the Institutional Review Board of Tokyo Women's Medi-

**Table 1** Baseline characteristics of study participants.

| Characteristic | Value |
|---|---|
| Patients, n | 41 |
| Age, y (median, IQR) | 6-15 (10, 9-12.5) |
| Male, n (%) | 29 (71) |
| BMI-z score, median (IQR) | 1.9 (-1-3.5) |
| Medical history of bronchial asthma, n (%) | 5 (12) |
| Using inhaled corticoid steroid, n (%) | 0 (0) |
| Medical history of allergic rhinitis, n (%) | 21 (51) |
| Using nasal drop corticoid steroid, n (%) | 0 (0) |
| Medical history of food allergy, n (%) | 4 (10) |
| Medical history of atopic dermatitis, n (%) | 7 (17) |
| No medical history of allergy, n (%) | 8 (19.5) |
| Smoker of patient's household, n (%) | 24 (59) |

BMI-z score, body mass index-z score; IQR, interquartile range.

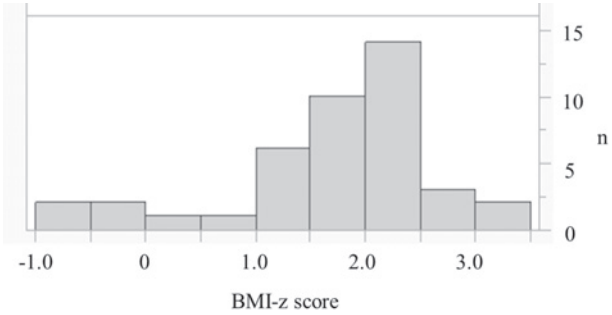


**Figure 1** Distribution of BMI-z score for the present study participants.
BMI-z score, body mass index-z score.

cal University in accordance with the Declaration of Helsinki (approval number: 130818).

## Results

### 1. Clinical and demographic characteristics

Forty-one participants, including 29 (70.7%) male, aged 6-15 years (median 10 years, interquartile range [IQR]: 9-12.5) were recruited. Participants' baseline characteristics are shown in **Table 1**; BMI-z score distribution is shown in **Figure 1**. BMI-z scores ranged from −1.0 to 3.5 (median 1.9). In five (12.2%) cases, the BMI-z score was ≥2.5, and these cases were classified as severe obesity. In 19 (46.3%) cases, the BMI-z score was ≥2.0, and these cases were classified as moderate obesity. Twenty-one (51.2%) patients had allergic rhinitis. Five (12.2%) patients had bronchial asthma, and none had undergone inhaled corticosteroid therapy. Twenty patients (48.8%) had an overlapping allergy disease. Eight patients (19.5%) had no allergic disease.

### 2. Low-FENO (<35 ppb) vs. high-FENO (≥35 ppb) groups

Thirty-three (80.5%) and eight (19.5%) participants were included in the low- and high-FENO groups, respectively (**Table 2**). The prevalence of allergic rhinitis was 48.5% (n=16) and 62.5% (n=5) in the low- and high-FENO groups, respectively (p=0.5). The median BMI-z score was 1.8 and 2.0 in the low- and high-FENO groups, respectively (p=0.7), indicating that BMI-z score was not associated with FENO. The median waist circumference was 81 cm and 87 cm in the low- and high-FENO groups, respectively (p=0.07).

The median B-Eos values were significantly higher in the high-FENO group (343/μL, IQR: 231-703/μL) than in the low-FENO group (184/μL, IQR: 80-279/μL) (p=0.004).

As for adipocytokines, only adiponectin levels significantly differed between the groups. The median levels of serum adiponectin were significantly lower in the high-FENO group (6.5 ng/mL) than in the low-FENO group (8.1 ng/mL) (p=0.0142) (**Table 2**). Leptin and PAI-1 levels did not differ significantly between the two groups (p=0.8 and p=0.6, respectively).

The median values of the MDA-LDL/LDL ratio as a marker of oxidative stress were 0.59 and 0.65 in the low-FENO and high-FENO groups, respectively (p=0.07). HDL cholesterol, LDL cholesterol, TG, AST, ALT, AST/ALT ratio, γ-GTP, uric acid, insulin, IgE, and high-sensitivity C-reactive protein levels did not differ significantly between the two groups.

### 3. Univariable correlation coefficient with FENO

In univariable correlation analyses, B-Eos (r=0.58, p<0.0001) and uric acid (r=0.34, p=0.03) were significantly correlated with FENO (**Table 3**). Adiponectin levels (r=−0.26, p=0.09) and leptin/adiponectin ratio (r=0.27, p=0.09) tended to correlate with FENO, but this finding did not meet the threshold for statistical significance. Scatter plot diagrams capturing univariate correlations between FENO and several explanatory variables are shown in **Figure 2**. The BMI-z score was not significantly correlated with FENO.

**Table 2** Comparison between the low-FENO (<35 ppb) and high-FENO (≥35 ppb) groups.

| | FENO <35 ppb, n=33 | FENO ≥35 ppb, n=8 | p-value |
|---|---|---|---|
| Age, (median) | 10 | 12 | 0.1 |
| Male, n (%) | 21 (64) | 8 (100) | 0.08 |
| Allergic rhinitis, n (%) | 16 (48) | 5 (62.5) | 0.5 |
| Bronchial asthma, n (%) | 1 (3) | 4 (50) | 0.03* |
| Food allergy, n (%) | 3 (9) | 0 (0) | 1.0 |
| Atopic dermatitis, n (%) | 5 (15) | 2 (25) | 0.6 |
| Smokers in patient's household, n (%) | 20 (60) | 4 (50) | 0.6 |
| BMI-z score, median (IQR) | 1.8 (1.3-2.7) | 2.0 (1.7-2.3) | 0.7 |
| Waist circumference (cm), median (IQR) | 81 (71-94) | 87 (85-104) | 0.07 |
| Waist circumference/height ratio, median (IQR) | 0.55 (0.51-0.63) | 0.59 (0.51-0.64) | 0.6 |
| Systolic blood pressure (mmHg), median (IQR) | 117 (106-123) | 111 (104-113) | 0.1 |
| Diastolic blood pressure (mmHg), median (IQR) | 66 (60-74) | 62.5 (60-84) | 0.9 |
| Body fat percentage (%), median (IQR) | 43 (38-47) | 44 (33-48) | 0.9 |
| IgE (U/mL), median (IQR) | 115 (42-221) | 207 (124-287) | 0.1 |
| Blood eosinophil counts (/μL), median (IQR) | 184 (80-279) | 343 (231-703) | 0.004* |
| Leptin (ng/mL), median (IQR) | 22 (12-31) | 20 (9-32) | 0.8 |
| Adiponectin (ng/mL), median (IQR) | 8.1 (6.4-10.3) | 6.5 (3.9-7.4) | 0.01* |
| Leptin/adiponectin ratio, median (IQR) | 2.7 (1.6-4.6) | 3.7 (1.3-9.1) | 0.5 |
| PAI-1 (ng/mL), median (IQR) | 30 (20-43) | 34 (20-46) | 0.6 |
| MDA-LDL cholesterol/LDL cholesterol ratio (mU/mg), median (IQR) | 0.59 (0.5-0.69) | 0.65 (0.62-0.70) | 0.07 |
| High-sensitivity CRP (μg/dL), median (IQR) | 792 (175-2,005) | 1,620 (355-4,408) | 0.2 |
| HDL cholesterol (mg/dL), median (IQR) | 55 (48-64) | 53 (40-62) | 0.4 |
| LDL cholesterol (mg/dL), median (IQR) | 94.8 (77-120) | 100.4 (77-138) | 0.6 |
| TG (mg/dL), median (IQR) | 71 (56-108) | 83.5 (58-122) | 0.6 |
| AST (U/L), median (IQR) | 25 (19-32) | 23 (28-29) | 0.7 |
| ALT (U/L), median (IQR) | 22 (16-48) | 36 (17-61) | 0.5 |
| AST/ALT ratio, median (IQR) | 0.9 (0.63-1.4) | 0.7 (0.43-1.0) | 0.2 |
| γ-GTP (U/L), median (IQR) | 17 (13-28) | 24 (14-46) | 0.2 |
| Uric acid (mg/dL), median (IQR) | 5.5 (4.7-6.5) | 6.3 (5.1-8.4) | 0.2 |
| Insulin (μIU/mL), median (IQR) | 10 (7.5-15.2) | 17 (7.3-18.6) | 0.5 |
| Blood glucose (mg/dL), median (IQR) | 95 (93-102) | 96 (88-97) | 0.6 |

FENO, fractional exhaled nitric oxide; BMI-z score, body mass index-z score; IQR, interquartile range; IgE, immunoglobulin E; PAI-1, plasminogen activator inhibitor-1; MDA-LDL, malondialdehyde-modified low-density lipoprotein; LDL, low-density lipoprotein; CRP, C-reactive protein; HDL, high-density lipoprotein; TG, triglycerides; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase.

### 4. Univariable correlation coefficient with B-Eos

In the univariable analysis, IgE (r=0.59, p<0.0001), adiponectin (r=−0.34, p=0.03), the leptin/adiponectin ratio (r=0.32, p=0.004), PAI-1 (r=0.42, p=0.006), TG (r=0.33, p=0.04), the AST/ALT ratio (r=−0.36, p=0.02), and γ-GTP (r=0.41, p=0.01) were significantly correlated with B-Eos (**Table 4**). Scatter plot diagrams capturing these associations are shown in **Figure 3**.

### 5. Logistic regression model for the high-FENO group

In the univariable analysis, B-Eos, adiponectin, leptin/adiponectin ratio, and urine acid were significantly associated with FENO (p< 0.1). The stepwise selection process revealed the combination of B-Eos and adiponectin as the best-matched logistic regression model for the high-FENO group. The AIC score was 29.52 and was lowest in case of the combination of B-Eos and adiponectin. In the multivariable logistic regression model, the odds ratios for B-Eos and adiponectin were 1.055 (95% confidence interval [CI] 1.0004-1.11) and 0.7 (95% CI 0.45-1.09) respectively (**Table 5**).

## Discussion

To our knowledge, this is the first study to reveal the association of adipocytokines and oxidative stress in bronchial asthma based on the measurement of FENO in Japanese children and adolescents with obesity.

**Table 3** Univariable correlations between FENO and explanatory variables.

| | r | p-value |
|---|---|---|
| BMI-z score | 0.09 | 0.6 |
| Waist circumference | 0.19 | 0.2 |
| Waist circumference/height ratio | 0.04 | 0.8 |
| Systolic blood pressure | -0.11 | 0.5 |
| Diastolic blood pressure | -0.004 | 0.9 |
| Body fat percentage | 0.03 | 0.8 |
| IgE | 0.15 | 0.3 |
| Peripheral blood eosinophil counts | 0.58 | <0.0001* |
| Leptin | -0.093 | 0.6 |
| Adiponectin | -0.26 | 0.09 |
| Leptin/adiponectin ratio | 0.27 | 0.09 |
| PAI-1 | 0.2 | 0.2 |
| MDA-LDL cholesterol/LDL cholesterol ratio | 0.26 | 0.1 |
| High-sensitivity CRP | 0.085 | 0.6 |
| HDL cholesterol | 0.002 | 0.9 |
| LDL cholesterol | 0.15 | 0.4 |
| TG | 0.04 | 0.8 |
| AST | -0.029 | 0.9 |
| ALT | 0.11 | 0.5 |
| AST/ALT ratio | -0.22 | 0.2 |
| γ-GTP | 0.17 | 0.3 |
| Uric acid | 0.34 | 0.03* |
| HbA1c | -0.096 | 0.6 |
| Insulin | 0.13 | 0.4 |
| Blood glucose | -0.042 | 0.8 |

FENO, fractional exhaled nitric oxide; BMI-z score, body mass index-z score; IgE, immunoglobulin E; PAI-1, plasminogen activator inhibitor-1; MDA-LDL, malondialdehyde-modified low-density lipoprotein; LDL, Low-density lipoprotein; CRP, C-reactive protein; HDL, high-density lipoprotein; TG, triglycerides; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase; HbA1c, hemoglobin A1c.

Notably, there was no significant correlation between FENO and BMI-z score or between B-Eos and BMI-z score. These results suggest that the degree of obesity assessed by body weight and height is not associated with eosinophilic inflammation of the airway and the whole body. Similar results have been reported in previous studies.[26, 27] For example, Santamaria et al. have reported no significant difference in FENO between children with obesity (mean FENO: 12.5 ppb, n=50) and children with normal weight (mean FENO: 10.8 ppb, n=50).[27]

In the present study, we have demonstrated that B-Eos values were significantly higher in the high-FENO (≥35 ppb) group than in the low-FENO group. Moreover, FENO and B-Eos levels were significantly correlated. FENO is known to reflect eosinophilic inflammation of the airway while B-Eos reflects eosinophilic inflammation of the whole body. Although FENO and B-Eos are considered independent risk factors for bronchial asthma, asthma attacks, and wheezing,[19] our findings suggests that systemic eosinophilic inflammation may associate with local eosinophilic inflammation of the airway.

Regarding adipocytokines, the serum adiponectin levels were significantly lower in the high-FENO (≥35 ppb) group than in the low-FENO group. Moreover, the univariable odds ratio of the serum adiponectin levels for high-FENO was 0.62 (95%CI 0.41-0.95), though the association was not independent with B-Eos. These results suggest that the serum adiponectin levels might associate with eosinophilic inflammation of the airway.

The significant negative univariate correlation between serum adiponectin levels and B-Eos suggests that a decrease in serum adiponectin levels is positively associated with eosinophilic inflammation of the whole body. Zhu et al. have previously demonstrated that adiponectin alleviates airway hyper-responsiveness, airway inflammation, and oxidative stress in a murine model of

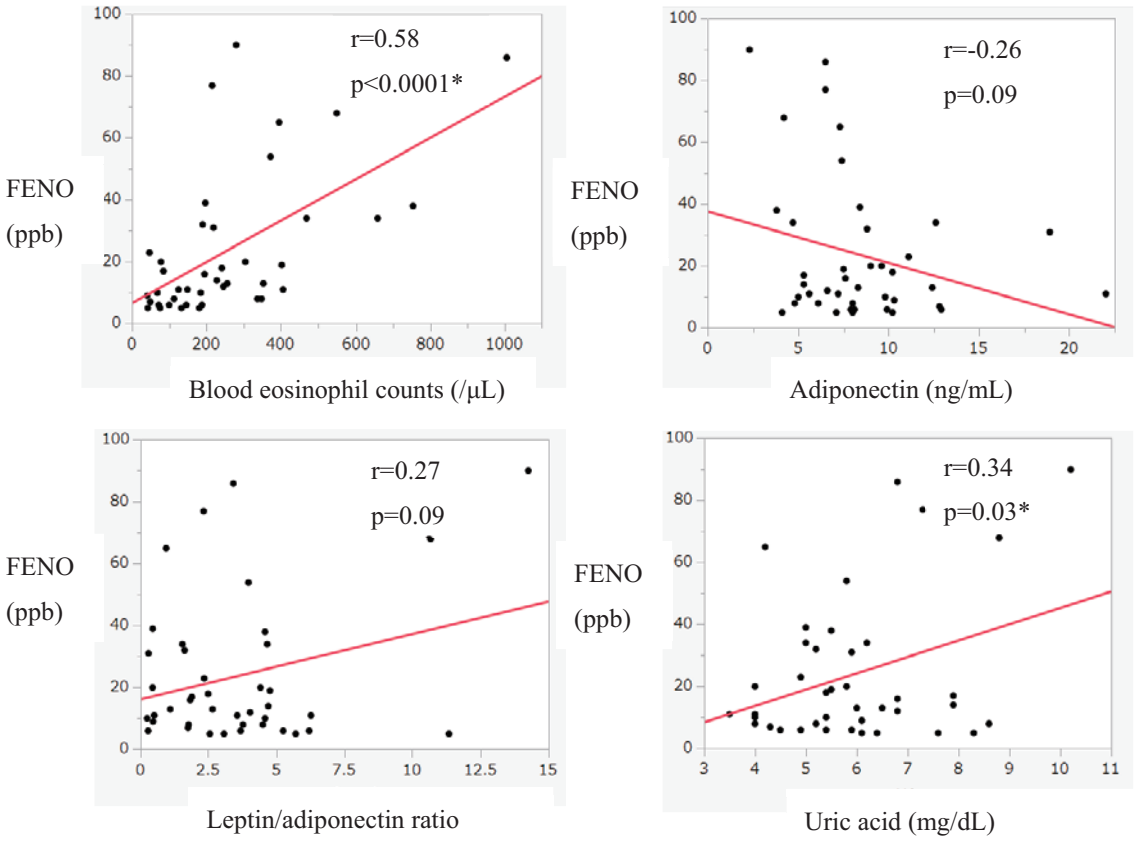


**Figure 2** Scatter plots illustrating univariate correlations between FENO and explanatory variables. FENO, fractional exhaled nitric oxide.

obesity-related asthma.[28] Although this mechanism has not been proved in human model, our result suggests similar mechanism exits in human.

Although increased serum leptin is a risk factor for bronchial asthma in adult women,[13] we did not find any relationship between serum leptin level and FENO. The likely reasons for this difference are as follows. First, the duration of high serum leptin levels might have affected the results. Second, no female subjects were included in the high-FENO group in our study; therefore, we cannot exclude the possibility that leptin may affect eosinophilic inflammation of the airway in females.

Although PAI-1 was not significantly related to FENO, it was significantly positively correlated with B-Eos suggesting that PAI-1 may influence systemic eosinophilic inflammation.

The MDA-LDL/LDL ratio had a significant positive correlation with FENO and B-Eos. This result indicates that oxidative stress may be positively associated with eosinophilic inflammation of the airway and of the whole body.

Visceral fat accumulation might induce changes in adipocytokines, such as adiponectin and PAI-1, and oxidative stress.[9, 15] Dekker et al. have reported that subcutaneous fat was not associated with FENO, while preperitoneal fat was positively associated with FENO in 6,178 children aged 6 years.[29] The decrease in serum adiponectin levels and the increase in systemic oxidative stress were associated with visceral fat accumulation.[30] We have demonstrated that waist circumference, which reflects visceral fat accumulation, tended to be higher in the high-FENO group. We have also revealed the influence of serum adiponectin levels on eosinophilic inflammation of the airway and the whole body. Additionally, there was a significant correlation between the MDA-LDL/LDL ratio and FENO. These data suggest that an association between visceral fat accumulation and eosinophilic inflammation of the airway and the whole body. The change in fat distribution, especially visceral fat accumulation in relation to obesity, may be one of the critical factors that links childhood obesity to the development of bronchial asthma in children and adolescents.

**Table 4** Univariable correlations between B-Eos and explanatory variables.

| | r | p-value |
|---|---|---|
| BMI-z score | 0.22 | 0.2 |
| Waist circumference | 0.2 | 0.2 |
| Waist circumference/height ratio | 0.2 | 0.2 |
| Systolic blood pressure | 0.054 | 0.7 |
| Diastolic blood pressure | 0.24 | 0.2 |
| Body fat percentage | 0.12 | 0.5 |
| IgE | 0.59 | <0.0001* |
| Leptin | 0.23 | 0.1 |
| Adiponectin | -0.34 | 0.03* |
| Leptin/adiponectin ratio | 0.32 | 0.004* |
| PAI-1 | 0.42 | 0.006* |
| MDA-LDL cholesterol/LDL cholesterol ratio | 0.33 | 0.04* |
| High-sensitivity CRP | 0.24 | 0.1 |
| HDL cholesterol | -0.078 | 0.6 |
| LDL cholesterol | 0.19 | 0.2 |
| TG | 0.33 | 0.04* |
| AST | 0.23 | 0.1 |
| ALT | 0.38 | 0.01* |
| AST/ALT ratio | -0.36 | 0.02* |
| γ-GTP | 0.41 | 0.01* |
| Uric acid | 0.21 | 0.2 |
| HbA1c | 0.17 | 0.3 |
| Insulin | 0.25 | 0.1 |
| Blood glucose | 0.049 | 0.8 |

B-Eos, blood eosinophil counts; BMI-z score, body mass index-z score; IgE, immunoglobulin E; PAI-1, plasminogen activator inhibitor-1; MDA-LDL, malondialdehyde-modified low-density lipoprotein; LDL, low-density lipoprotein; CRP, C-reactive protein; HDL, high-density lipoprotein; TG, triglycerides; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase; HbA1c, hemoglobin A1c.

Our study had several limitations. First, because of the small sample size, type II errors cannot be excluded, and our study lacks statistical power. Second, we could not detect a clear relationship between eosinophilic inflammation and the duration of obesity or the change in visceral fat, adipocytokine, and oxidative stress because we examined single factors in this study. Third, although we used waist circumference, waist circumference/height ratio, adiponectin, PAI-1, ALT, AST/ALT ratio, and γ-GTP as markers of visceral fat, we had not performed X-ray examinations to quantify visceral fat accumulation. As a result, we cannot draw meaningful conclusions regarding an association between eosinophilic inflammation and visceral fat accumulation. Finally, the high-FENO group did not include any female participants. Because our sample size was small and this was a cross-sectional study, our findings cannot be used to determine whether the reported gender differences resulted from coincidence or adipocytokine profiles.

## Conclusions

In conclusion, eosinophilic inflammation might be associated with a decrease in serum adiponectin levels which might be induced by visceral fat accumulation. The presence of obesity alone is not a risk factor for eosinophilic inflammation of the airway. Notably, visceral fat accumulation might be a major cause of eosinophilic inflammation. To elucidate the link between childhood obesity and bronchial asthma, future research should examined body fat distribution in addition to body weight and BMI-z score among participants with obesity.

## Acknowledgements

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The authors' contributions are as follows: AN contributed to the study design, data collection, data analysis, generation

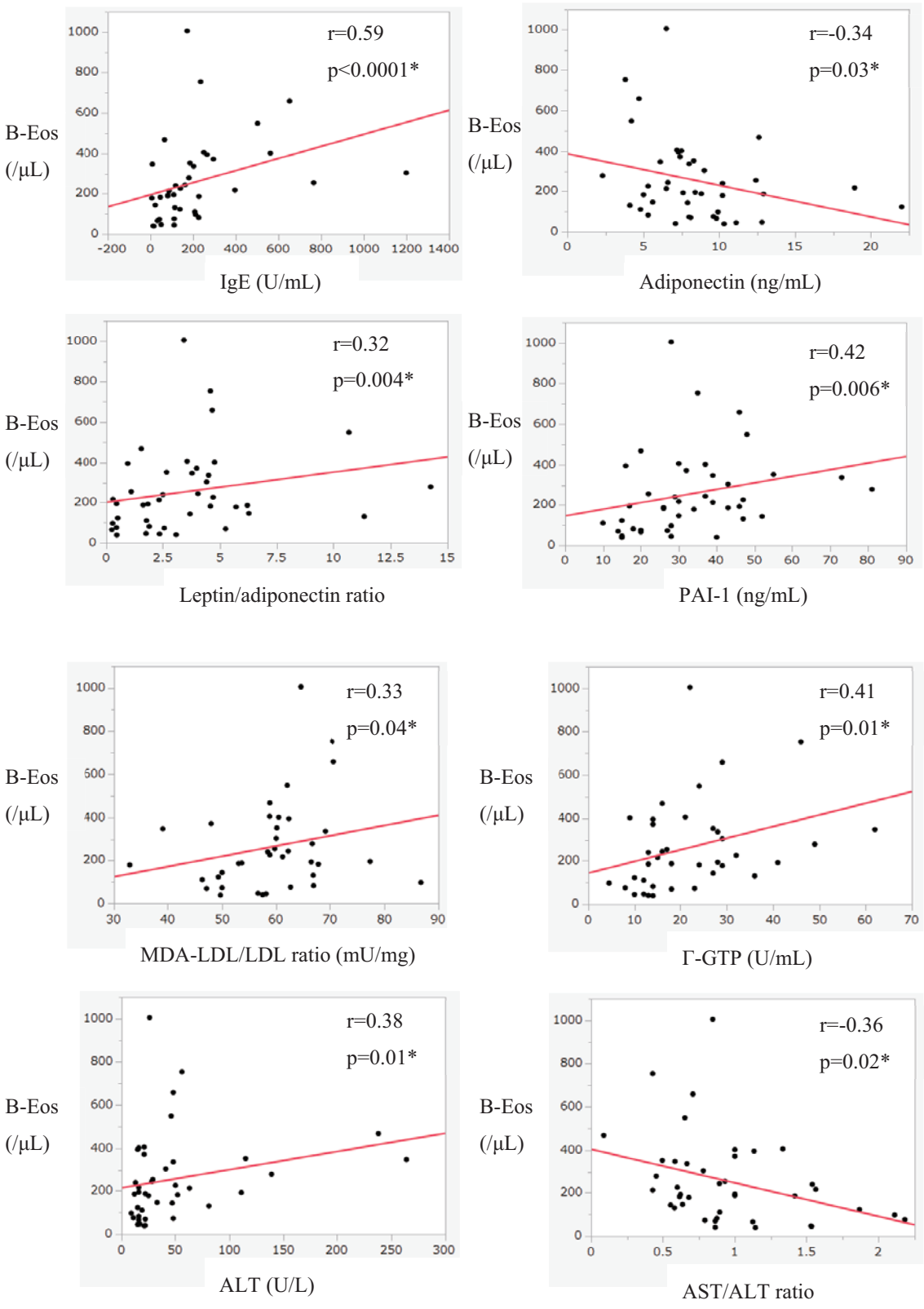


**Figure 3** Scatter plots illustrating univariate correlations between B-Eos and explanatory variables. B-Eos, blood eosinophil counts; IgE, immunoglobulin E; PAI-1, plasminogen activator inhibitor-1; MDA-LDL, malondialdehyde-modified low-density lipoprotein; LDL, low-density lipoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

**Table 5** Logistic regression model for the high-FENO (≥35 ppb) group.

| | Univariable | | Multivariable | |
|---|---|---|---|---|
| | Odds ratio | 95% CI | Odds ratio | 95% CI |
| B-Eos (/10 μL) | 1.068 | 1.015-1.12 | 1.055 | 1.0004-1.11 |
| Adiponectin | 0.62 | 0.41-0.95 | 0.7 | 0.45-1.09 |

FENO, fractional exhaled nitric oxide; CI, confidence interval; B-Eos, blood eosinophil counts.

of figures, and the development of the manuscript. OT contributed to the study design, data analysis, and the development of the manuscript. All authors were involved in discussions about the results and in writing the manuscript and have approved this manuscript.

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