

MR. WENLONG LIU (Orcid ID : 0000-0003-1296-5144)

Article type : Original

**Association of leptin with disease severity and inflammation
indicators in Chinese obese children with allergic rhinitis**

Wenlong Liu, PhD^a, Qingxiang Zeng, PhD^a, Lifeng Zhou, MD^a,
Renzhong Luo, MD^a, Haipeng Dong, MD^b

From ^a Department of Otolaryngology, Guangzhou Women and Children's Medical
Center, Guangzhou Medical University, Guangzhou, China.

^b Department of Children Health Care, Guangzhou Women and Children's Medical
Center, Guangzhou Medical University, Guangzhou, China.

Running title: leptin in allergic rhinitis children

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/pai.12856

This article is protected by copyright. All rights reserved.

*Correspondence to : Wenlong Liu and Renzhong Luo, Guangzhou Women and

Children's Medical Center, No 9. Jinsui Road, Guang Zhou, 510623.

Tel:008613751872495. Fax:00862038076166.

Email: lwl20103@163.com and renzhongluo@21cn.com

Word count: 2270; Number of Tables:6.

Association of leptin with disease severity and inflammation indicators in Chinese obese children with allergic rhinitis

WL Liu, QX Zeng, LF Zhou, RZ Luo, HP Dong,

Abstract

Background: The prevalence of both obesity and allergic diseases in children has increased over the last several decades. However, the direct relationship between diverse allergic diseases and obesity has varied in different studies. Therefore, we aimed to examine the effect of obesity on the incidence and severity of allergic rhinitis (AR) and the possible key inflammation mediators during AR.

Methods: A total of 3,126 healthy students (without chronic diseases) were recruited from 14 randomly selected secondary schools in Guangzhou, China. Body mass index (BMI), waist circumference (WC), waist-to-height ratio (WHR) and body fat percentage (PBF) were measured and compared. The effect of obesity indicators and leptin level (exposures) on the incidence (primary outcome) and severity of AR (secondary outcomes) were analyzed. Inflammatory markers were detected and compared among groups.

Results: The symptom score (9.5 ± 3.1 vs 8.2 ± 3.5 , $P < 0.05$) and medication score (3.6 ± 1.6 vs 2.9 ± 1.8 , $P < 0.05$) were significantly higher in obese children with AR than in non-obese children with AR. After adjusted for potential confounders, multiple linear regression analysis showed that the serum leptin concentration was significantly correlated with the levels of T help (TH) 2 cytokines (coefficient, 0.48[95%CI, 0.05-0.91]), TH17 cytokines (coefficient, 0.39[95%CI, 0.11-0.89]), regulatory T cell cytokines (IL-10, coefficient, -0.43[95%CI, -0.02-0.65]; TGF-beta, coefficient, -0.65[95%CI, -0.06-1.35]) in patients with AR.

Conclusions: Our study shows that obesity exacerbates inflammation and contributes to disease severity in AR. Our study provides evidence that leptin was involved in enhanced TH inflammation as well as the accumulation and activation of inflammatory cells in obese children with AR.

Key words: leptin, obesity, children, allergic rhinitis

This article is protected by copyright. All rights reserved.

Introduction

Over the past several decades, the prevalence of obesity in children has increased concurrently with allergic diseases, such as asthma, allergic rhinitis (AR) and atopic dermatitis (AD).^{1,2} However, the direct relationship between diverse allergic diseases and obesity has varied in different studies³⁻⁵. Specifically, numerous studies have shown a relationship between an increased body mass index (BMI) and asthma, especially in girls.⁶⁻⁸ Kusunoki's study suggested that childhood obesity positively correlates with AD prevalence and severity, whereas it negatively correlates with AR and allergic conjunctivitis (AC) prevalence, especially among boys.⁹ Conversely, Braback's study did not find a significant correlation between BMI and AC.¹⁰

The underlying mechanisms for the connection between obesity and atopy remain unclear. Obesity is a low-grade systemic inflammatory state characterized by altered levels of adipokines, such as adiponectin, adipon, resistin^{11,12} These immunological changes may decrease immunological tolerance to allergens and cause a shift toward a T helper 2 (TH2) cell immune response, which increases the risk of atopic diseases.¹³ However, not all evidence supports this hypothesis, some data suggest that obesity is predominantly or exclusively associated with non-atopic asthma.^{14,15}

BMI is commonly used in epidemiologic studies, but it cannot evaluate individuals who are overweight because of high muscle mass. Alternatively, the waist circumference (WC) and waist-to-height ratio (WHR) closely reflect the amount of

visceral fat deposits, which metabolically differ from other body fat.¹⁶ Moreover, the WC and WHR are more robust than the BMI across ethnic groups, age groups and between men and women.¹⁷⁻¹⁹ Nevertheless, the body fat percentage (PBF) is the only body measurement that directly calculates a person's relative body composition without regard to height and weight. Therefore, we suppose that a combination of those above indicators may more subjectively reflect obesity.

Few studies have examined the relationship between AR and obesity and the findings of these studies have been inconsistent, especially in Chinese children. In this study, we aimed to examine the effect of obesity on the incidence and severity of perennial AR using different indicators and the possible key inflammation mediators during the progression of AR in children.

Methods

Study population and the inclusion and exclusion criteria

This study was a cross-sectional, population-based survey conducted between January and December 2016. A total of 3,126 subjects (7-11 years old) were recruited from 14 randomly selected secondary schools in Guangzhou, China. Children with chronic diseases (e.g. asthma, malnutrition, anatomic malformation of the respiratory system, chronic lung disease, heart disease, gastro-esophageal reflux disease, cystic

fibrosis) and those with a history of chronic drug use (e.g. antiepileptics, immune-suppressive agents) were excluded from the study. The study was performed with the approval of the local ethics committee and with the parents' written informed consent.

Baseline information collection

Baseline information, such as sex, age, parental allergic history, passive smoking history and serum lipid level were collected. As described previously^{20,21}, passive smoke exposure was defined as having one or both parents who smoke or being exposed to tobacco smoke or to a person who smokes indoors. Specifically, the exposed child was in the same room with a smoker for at least an hour a day for 12 consecutive months or more.

Anthropometric measurements

Body weight and height were measured, with the subject lightly clothed and bare-footed to calculate BMI in kilograms per square meter. Being overweight was defined as a BMI greater than the local age- and gender- specific 85th percentile (or 25kg/m², whichever was smaller) and obesity was defined as a BMI greater than the 95th percentile (or 30kg/m², whichever was smaller) and the rest were normal weight. These percentile standards were established by a local population survey.²² BMI

z-score were obtained using the CDC Epi Info nutritional module to adjust for age and gender associated variation in BMI percentiles. The waist circumference was taken midway between the lowest rib and the superior border of the iliac crest at the mid-axillary line to the nearest 0.1 cm.²³ The WHR was calculated by dividing the waist circumference by the height. The Tanita BC-418MA (Tokyo, Japan) segmental body composition analyzer was used to measure PBF by means of bioelectrical impedance analysis.

Diagnosis of AR

The diagnosis of AR was made based on history, clinical examination, skin prick test and specific IgE measurements according to the Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines.²⁴ The atopic status was evaluated by a skin prick test or the detection of IgE (Phadia) specific to common inhalant allergens (dust mites, pets, cockroach, pollen, etc.). Specific IgE values greater than or equal to 0.35 kIU/L were considered positive. Perennial AR children were confirmed according to the duration of symptoms and the type of allergens.

Symptom and medication score

A total nasal symptom score (TNSS) and a visual analog scale (VAS) score were calculated for the evaluation of severity in children. Individual nasal symptoms including sneezing, rhinorrhea, itchy nose, and nasal congestion were recorded on a 0 to 3 scale (0, none; 1, mild; 2, moderate; and 3, severe) and the total nasal symptom score (0-12) was summed. The visual analog scale score was evaluated by a doctor on a 0- to 10-cm visual analog scale, with 0 cm being “no complaints whatsoever” and 10 cm being “worst imaginable complaints.”

The patients were only allowed to use the following drugs: antihistamine (5 mg levocetirizine tablets), intranasal corticosteroid (mometasone), topical ocular antihistamine drops (ebastine) and a 4 mg tablet of methylprednisolone. The patients recorded the use of medications on the diary card, and a specific weekly score was assigned (1 point: used only nasal corticosteroids or eye drops at a minimum of per 1 day per week; 2 points: the previous therapy plus one tablet of levocetirizine at a minimum of per 1 day; 3 points: the previous therapies plus one 4 mg tablet of methylprednisolone at a minimum of per 1 day).

The nasal symptoms and medication scores were recorded and averaged in an 8-week observation period.

Serum lipid profile and inflammatory markers

Six milliliters of venous blood was collected from each of the 200 participants (50 obese children, 50 normal children, 50 obese children with AR, 50 non-obese children with AR) after overnight fasting for at least 8 hours. The serum samples were taken both during and out of allergen season, respectively. Routine blood tests were also performed. The serum concentration of high-sensitivity C-reactive protein (hsCRP) was measured by chemiluminescence immunoassays using an Immulite 2000 Analyzer. The sensitivity of this hsCRP assay was 0.1 mg/L.

After centrifuging at 1000g 4°C for 15 minutes, the serum samples were separated and stored at -80°C until use in the assay. The serum levels of leptin, IFN- γ , IL-5, IL-10, IL-17 and TGF-beta were measured in duplicate using enzyme-linked immuno sorbent assay (ELISA) kits (R&D, USA) according to the manufacturer's instructions. The total serum IgE level was measured with an ELX-800 system. Eosinophil cationic protein (ECP) was detected by Unicap system. Routine blood test was also performed. The detection limits of the assays were as follows: leptin (22 pg/mL), IFN- γ (12.5 pg/mL), IL-5 (7.8 pg/mL), IL-10 (3.9 pg/mL), IL-17 (15 pg/mL), TGF-beta (14.5 pg/mL) and ECP (2.0 ng/mL).

Statistical analysis

The obesity indicators and leptin level were exposures in this study. AR diagnosis and AR severity were primary and secondary outcomes, respectively. The age, sex and passive smoke history were examined as potential confounders. All the dependent variables were continuous. Normally distributed data are presented as mean $\pm$ SD whereas skewed data are presented as median. The study groups were compared by analysis of variance. Between-group comparisons were conducted using a Bonferroni post hoc analysis to adjust for multiple comparisons.

In order to examine the association between obesity indicators and disease severity, leptin level and inflammatory mediators, leptin level and disease severity, multiple linear regression analysis was performed using obesity indicators or leptin as the dependent variable. Correlation between leptin and obesity indicators was analyzed by Pearson's correlation test. All linear regressions were performed in a combined model after proper adjustments for age, gender, passive smoke history. Data were interpreted as standardized coefficients with 95% CIs. A P value of less than 0.05 was defined as significant. SPSS17.0 statistical package was used for Statistical analysis.

Results

Characteristics of subjects and prevalence of AR

The demographic characteristics of participants are summarized in Table 1 as obesity defined based on BMI calculation. As expected, BMI significantly correlated with the other three obesity indicators, although the degree of correlation and the slope of the regression coefficient varied (Table E1).

The prevalence of being overweight or obese was comparable between the AR and normal weight group and did not differ by gender. The total nasal symptom score, individual nasal symptom score, VAS score and medication score were significantly higher in obese children with AR than in non-obese children with AR ($P<0.05$) (Table 1).

We also found that serum lipid level, TIG level and percentage of passive smoke were higher in obese children with AR than in non-obese children with AR ($P<0.05$). Parental allergy history and allergen ratio are not different between groups.

Relationship between obesity indicators and disease severity

Regression analysis showed that BMI, PBF and WC were associated with increased TNSS score, individual score, VAS score and medication score, whereas WHR score was associated with increased odds of TNSS score and blockage score (Table 2).

Relationship between leptin and Th-response and other inflammatory markers

Our results showed that the serum leptin, white cell count (WCC), CRP, IgE, eosinophil count, neutrophil count, basophil count, and ECP concentrations were significantly increased in children with AR compared with normal controls, especially in children with obesity ($P<0.05$) (Table 3). Regression analysis showed that the serum leptin concentration was associated with increased odds of eosinophil count (coefficient, 0.489[95%CI, 0.15-1.54], $P<0.05$), ECP (coefficient, 0.56[95%CI, 0.03-0.97], $P<0.05$), WCC (coefficient, 0.13[95%CI, 0.01-0.43], $P<0.05$), CRP (coefficient, 0.51[95%CI, 0.03-1.23], $P<0.05$), IL-5 (coefficient, 0.48[95%CI, 0.05-0.91], $P<0.05$), IL-17 (coefficient, 0.39[95%CI, 0.11-0.89], $P<0.05$) and decreased odds of IL-10 (coefficient, -0.43[95%CI, 0.02-0.65], $P<0.05$) and TGF-beta(coefficient, -0.65 [95%CI, -0.06-1.35], $P<0.05$) in patients with AR (Table 4).

Relationship between leptin and obesity indicators as well as disease severity

We also found that the serum leptin concentration was correlated with BMI, WC and PBF for both girls and boys (Table 5). Regression analysis showed the serum leptin levels were associated with TNSS score, individual score, VAS score and medication score for both girls and boys (Table 6).

Discussion

This study is the first to examine the relationship between obesity indicators and the incidence and disease severity of perennial AR in a large cohort of children from the southern China mainland. We found that obesity increases the severity of perennial AR, and that this process was correlated with leptin.

Few studies have examined the relationship between AR and obesity and their findings have been inconsistent, especially in Chinese children. Specifically, several investigations have shown a positive association between BMI and atopy, AR or AC, especially among female young adults and children.²⁵ For example, Huang found a higher prevalence of atopy among girls in the highest BMI quintile, whereas Luo et al found that obesity was significantly associated with the presence of atopic dermatitis and atopic rhinitis in both genders.^{26,27} However, few studies investigated the correlation between AR and other obesity indicators (PBF, WC, WHR). In our study, the results showed that BMI correlated well with other measures of adiposity. Our data showed that the prevalence of being overweight or obese was comparable between AR and normal for all four obesity indicators, suggesting that susceptibility to AR was not related to obesity. Interestingly, our results showed that the prevalence of being overweight or obese was comparable between AR and normal even when gender was considered. Therefore, studies of large samples and multiple centers are needed to further validate our results.

Previous studies supported that obese children with AR exhibit more severe clinical symptoms.²⁸ To exclude the effect of other drugs on the symptom scores during 8-week observation period, the patients were only allowed to use the drugs we designated. Accordingly, our data for the first time showed that higher BMI, PBF and WC were significantly correlated with higher individual nasal symptom scores, total nasal symptom scores and medication score, whereas WHR was correlated with TNSS and blockage score regardless of gender. These results suggested that obesity exacerbate inflammation in the setting of AR. Therefore, we further tested and compared different inflammatory markers between obese and non-obese children with AR.

The adipokines are cytokines secreted by adipose tissue, such as leptin, adiponectin, adipisin, resisitin and so on. Several adipokines have been previously associated with an increase of AR symptoms¹². However, we focus only on leptin in this study since leptin has been reported to be involved in regulation of T cells. Leptin, which is released by adipocytes and was first described to regulate body weight, also exerts biologic functions in immune responses. Laboratory studies have indicated that the overall effect of leptin on T memory cells is to increase TH1 responses and decrease TH2 responses. However, recent studies also suggested that the expression of leptin is increased in allergic diseases (such as asthma and AR) and related to the severity and control of disease.²⁸⁻³⁰ To exclude the influence of respiratory system, nutritional disease or the drug use on serum leptin level, we ruled out children as described in the methods part. Interestingly, our results showed that

This article is protected by copyright. All rights reserved.

Accepted Article

serum leptin was related to enhanced TH2/17 inflammation and inhibited Treg function in obese children with AR. Since leptin has been reported to increase the TH1 responses and decrease the TH2 responses, we hypothesized that the direct effect of leptin on the TH1 response may be inhibited or weakened by other mediators. Moreover, the pathway and molecular mechanism involved in leptin-mediated TH inflammation also require further investigation.

Our study also showed that the leptin level was related to the eosinophil and WCC numbers as well as the levels of IgE, ECP, suggesting that leptin is involved in the accumulation and activation of inflammatory cells to enhance the allergic reaction. Similarly, previous study also suggested leptin was involved in accumulation and infiltration of eosinophils¹². As for other markers, the effect of leptin on them needed further exploration. Our results also suggested that serum leptin levels correlated significantly with disease severity, which is consistent with previous study.²⁸

Our study was a single-center study and focused on 7-12-year-old children. However, this recruitment of specific population reduces the effect caused by selection bias. Besides, we also examined gender as a confounder to reduce the influence of misclassification bias. There were still some limitations in our study. First, only perennial AR patients were discussed. Second, mechanism study was also not discussed in this study. Third, we examined age, sex and passive smoke history as potential confounders, but there are numerous variables we cannot measured one by one. For example, we exclude children with history of asthma and eczema to reduce

their effect on expression of inflammatory markers. Therefore, Further prospective studies with large samples are needed to find out more related variables.

In sum, our study provides evidence to show that leptin is involved in TH 2/17 inflammation and the accumulation and activation of inflammatory cells in obese children with AR. However, the exact mechanism underlying this effect requires further exploration in studies of large samples involving both in vivo and in vitro experiments.

Acknowledgement

This study was supported by grants from the National Natural Science Grant of China (No.81500772), the Guangdong Province Natural Science Grant (No.2014A030310216) and the Pearl River S&T Nova Program of Guangzhou (No. 201710010085).

Conflict of interest: None.

References

1. Baumann S, Lorentz A. Obesity-a promoter of allergy? *Int Arch Allergy Immunol* 2013;162:205-13.
2. Boulet LP. Obesity and atopy. *Clin Exp Allergy* 2015;45:75-86.
3. Han YY, Forno E, Gogna M, Celedón JC. Obesity and rhinitis in a nationwide study of children and adults in the United States. *J Allergy Clin Immunol*. 2016 May;137(5):1460-5.
4. Quek YW, Sun HL, Ng YY, Lee HS, Yang SF, Ku MS, et al. Associations of serum leptin with atopic asthma and allergic rhinitis in children. *Am J Rhinol Allergy*. 2010;24(5):354-8.
5. Sidell D, Shapiro NL, Bhattacharyya N. Obesity and the risk of chronic rhinosinusitis, allergic rhinitis, and acute otitis media in school-age children. *Laryngoscope*. 2013 Oct;123(10):2360-3.
6. Han YY, Forno E, Celedon JC. Adiposity, fractional exhaled nitric oxide, and asthma in U.S. children. *Am J Respir Crit Care Med* 2014;190:32-9
7. Forno E, Acosta-Perez E, Brehm JM, et al. Obesity and adiposity indicators, asthma, and atopy in Puerto Rican children. *J Allergy Clin Immunol* 2014;133:1308-14,1314.e1-5
8. Holguin F, Bleeker ER, Busse WW, et al. Obesity and asthma: an association modified by age of asthma onset. *J Allergy Clin Immunol* 2011;127:1486-93.e2
9. Kusunoki T, Morimoto T, Nishikomori R, et al. Obesity and the prevalence of allergic diseases in schoolchildren. *Pediatr Allergy Immunol* 2008;19:527-534
10. Braback I, Hjertqvist A, Rasmussen F. Body mass index, asthma and allergic rhinoconjunctivitis in Swedish conscripts-a national cohort study over three decades. *Respir Med* 2005;99:1010-1014.

11. Fantuzzi G. Adipose tissue, adipokines, and inflammation. *J Allergy Clin Immunol* 2005;115:911-919.
12. Ciprandi G, Caimmi D, Raschetti R, Miraglia Del Giudice M, Salpietro C, Caimmi S, Castellazzi AM. Adipokines and their role in allergies. *Int J Immunopathol Pharmacol*. 2011 Oct;24(4 Suppl):13-6.
13. Hersoug LG, Linneberg A. The link between the epidemics of obesity and allergic diseases: does obesity induce decreased immune tolerance? *Allergy* 2007; 62:1205-1213.
14. Appleton SL, Adams RJ, Wilson DH, Taylor AW, Ruffin RE. Central obesity is associated with nonatopic but not atopic asthma in a representative population sample. *J Allergy Clin Immunol* 2006;118:1284-1291.
15. Chen Y, Dales R, Jiang Y. The association between obesity and asthma is stronger in nonallergic than allergic adults. *Chest* 2006;130:890-895.
16. Arner P. Not all fat is alike. *Lancet* 1998;351:1301-2.
17. Ashwell M, Hsieh SD. Six reasons why the waist-to-height ratio is a rapid and effective global indicator for health risks of obesity and how its use could simplify the international public health message on obesity. *Int J Food Sci Nutr* 2005;56:303-7.
18. Hadaegh F, Zabetian A, Harati H, Azizi F. Waist/height ratio as a better predictor of type 2 diabetes compared to body mass index in Tehranian adult men-a 3.6-year prospective study. *Exp Clin Endocrinol Diabetes* 2006;114:310-5.
19. Janssen I, Katzmarzyk PT, Ross R. Waist circumference and not body mass index explains obesity-related health risk. *Am J Clin Nutr* 2004;79:379-84.
20. Oberg M, Jaakkola MS, Woodward A, Peruga A, Prüss-Ustün A. Worldwide burden of disease from exposure to second-hand smoke: a retrospective analysis of data from 192 countries. *Lancet*. 2011;377(9760):139-46.

21. McLachlan CR, Poulton R, Car G, Cowan J, Filsell S, Greene JM, Taylor DR, Welch D, Williamson A, Sears MR, Hancox RJ. Adiposity, asthma, and airway inflammation. *J Allergy Clin Immunol*. 2007 Mar;119(3):634-9.
22. Liu W, Liu W, Lin R, et al. Socioeconomic determinants of childhood obesity among primary school children in Guangzhou, China. *BMC Public Health* 2016 Jun 8;16:482.
23. Ng VW, Kong AP, Choi KC, et al. Use of BMI and waist circumference to predict clustering of cardiovascular risk factors in Hong Kong Chinese adolescents. *Obesity* 2007;15:1-10.
24. Bousquet J, Khaltayev N, Cruz AA, et al. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). *Allergy* 2008;63:8-160.
25. Kilpelainen M, Eerho EO, Helenius H, Koskenvuo M. Body mass index and physical activity in relation to asthma and atopic diseases in young adults. *Respir Med* 2006;100:1518-25.
26. Huang SL, Shiao G, Chou P. Association between body mass index and allergy in teenage girls in Taiwan. *Clin Exp Allergy* 1999;29:323-9
27. Luo X, Xiang J, Dong X, et al. Association between obesity and atopic disorders in Chinese adults: an individually matched case-control study. *BMC Public Health* 2013;13:12.
28. Hsueh KC, Lin YJ, Lin HC, Lin CY. Serum leptin and adiponectin levels correlate with severity of allergic rhinitis. *Pediatr Allergy Immunol* 2010;21:e155-e159.
29. Newson RB, Jones M, Forsberg B, Janson C, Bossios A, Dahlen SE, et al. The association of asthma, nasal allergies, and positive skin prick tests with obesity, leptin, and adiponectin. *Clin Exp Allergy*. 2014;44(2):250-60.

30.Ahmadizar F, Vijverberg SJ, Arets HG, de Boer A, Lang JE, Kattan M. Childhood obesity in relation to poor asthma control and exacerbation: a meta-analysis. Eur Respir J. 2016;48(4):1063-1073.

Table 1 Demographic characteristic of AR children and normal controls as obesity defined based on BMI calculation.

	AR (578)			Control (2548)		
	Normal	Overweight	Obesity	Normal	Overweight	Obesity
Number (%)	354(61.2%)	161(27.8%)	63(11%)	1593(62.5%)	712(27.9%)	243(9.6%)
Sex, male (%)	173(48.8%)	83(51.5%)	33(52.3%)	812(50.9%)	342(48%)	125(51.4%)
Age	9.4±2.8	9.7±2.1	8.4±2.2	9.5±2.6	9.6±2.3	9.1±2.1
Obesity measures						
BMI z score	0.4±0.2	1.5±0.3	2.1±0.2* [#]	0.3±0.1	1.6±0.2	2.3±0.1*
PBF	0.25±0.12	0.32±0.13	0.45±0.17* [#]	0.27±0.14	0.35±0.11	0.42±0.15*
WHR	0.83±0.05	0.87±0.04	0.96±0.06* [#]	0.83±0.05	0.87±0.04	0.96±0.06*
WC	61.2±10.1	72.5±14.3	83.6±15.5* [#]	61.2±10.1	72.5±14.3	83.6±15.5*
Serum lipid						
TC	3.95±0.34	4.02±0.38	4.35±0.26* [#]	3.87±0.28	4.11±0.34	4.31±0.18*
LDL	1.61±0.26	2.02±0.31	2.25±0.34* [#]	1.64±0.22	2.11±0.28	2.27±0.24*
HDL	1.25±0.23	1.21±0.19	1.14±0.15* [#]	1.27±0.19	1.20±0.15	1.12±0.11*
TGC	0.82±0.35	1.01±0.28	1.24±0.34* [#]	0.76±0.31	1.03±0.25	1.27±0.29*
sIgE to						
D1 (%)	75.2	72.1	73.4	0	0	0
D2 (%)	66.2	58.3	60.2	0	0	0
Cockroach (%)	12.3	10.9	11.3	0	0	0

Cat (%)	5.3	6.2	5.8	0	0	0
Dog (%)	7.4	8.1	6.9	0	0	0
Others (%)	2.3	2.6	2.2	0	0	0
TIgE (kIU/L)	323±167	417±213	467±235* [#]	135±52	126±46	143±39
Passive smoke (%)	3.2	6.5	10.1* [#]	4.2	7.4	9.3*
Parental allergy (%)	23	31	28	12	14	19
TNSS score	7.2±3.1	8.2±3.5	9.5±3.1 [#]	0	0	0
Runny nose	1.9±0.8	2.1±0.9	2.4±0.6 [#]	0	0	0
Sneezing	1.8±0.7	2.0±0.6	2.3±0.5 [#]	0	0	0
Blockage	2.1±0.8	2.2±0.6	2.5±0.6 [#]	0	0	0
Itching	1.7±0.5	1.9±0.5	2.2±0.7 [#]	0	0	0
VAS score	5.6±1.4	6.2±2.3	7.3±1.8 [#]	0	0	0
Medication score	2.4±1.2	2.9±1.8	3.6±1.6 [#]	0	0	0

Data presented as mean ± SD. AR, allergic rhinitis, BMI, body mass index. PBF, body fat percentage. WC, waist circumference, WHR, waist-to-height. TC, Total Cholesterol. LDL, low-density lipoprotein. HDL, high-density lipoprotein. TGC, Triglyceride. D1, Dermatophagoïdes pteronyssinus. D2, Dermatophagoïdes farinae. TNSS, total nasal symptom score. VAS, visual analog scale.

* compared with normal control group, P<0.05.

[#] compared with AR without obesity group, P<0.05.

	BMI		PBF		WC		WHR	
	SC	Adjusted R ²	SC	Adjusted R ²	SC	Adjusted R ²	SC	Adjusted R ²
TNSS score	0.71(0.2 to 1.43)	0.45*	0.56(0.3 to 1.21)	0.41*	0.77(0.3 to 1.77)	0.39*	0.58(0.1 to 1.16)	0.51*
Runny nose	0.62(0.1 to 0.93)	0.42*	0.53(0.2 to 0.96)	0.39*	0.62(0.1 to 0.93)	0.51*	0.61(0.01 to 0.72)	0.03
Sneezing	0.32(0.01 to 0.8)	0.35*	0.38(0.05 to 0.7)	0.43*	0.41(0.09 to 0.7)	0.39*	0.22(0.02 to 0.9)	0.11
Blockage	0.46(0.01 to 0.8)	0.58*	0.48(0.2 to 0.84)	0.62*	0.49(0.2 to 1.23)	0.61*	0.24(0.02 to 0.96)	0.58*
Itching	0.22(0.1 to 0.65)	0.35*	0.29(0.05 to 0.78)	0.41*	0.11(0.01 to 0.54)	0.39*	0.26(0.1 to 0.72)	0.05
VAS score	0.45(0.31 to 1.2)	0.54*	0.41(0.21 to 1.1)	0.47*	0.38(0.09 to 1.1)	0.42*	0.46(0.22 to 1.3)	0.02
Medication score	0.29(0.03 to 0.7)	0.38*	0.21(0.05 to 0.8)	0.31*	0.31 (0.01 to 0.8)	0.31*	0.18(0.01 to 0.5)	0.03

Regression analysis was performed after proper adjustment for age, gender and passive smoke history. * $P < 0.05$.

SC, standardized coefficient (95% CI). BMI, body mass index. PBF, body fat percentage. WC, waist circumference, WHR, waist-to-height. TNSS, total nasal symptom score. VAS, visual analog scale.

Table 3 Comparison of inflammatory cells and markers among different groups

Groups	Non-Obese AR	Obese AR	Obese control	Normal control
Eosinophil (count/mm ³)	134(60-1180) *	213(90-1640) *#	73(11-399) *	55(5-205)
Neutrophil (count/mm ³)	8500(7000-15500)	12300(8000-24100) #	6500(4000-16100)	7800(3800-14300)
Basophil (count/mm ³)	65(10-210) *	156(60-360) #	11(5-31)	8(4-18)
Monocytes (count/mm ³)	223(60-2500)	252(90-1800)	211(60-2190)	231(60-2120)
Lymphocyte (count/mm ³)	7900(3200-15600)	7300(4100-17100)	8600(3300-16600)	8100(2800-15300)
ECP (ng/mL)	39.1(4.0-131.0) *	57.2(39.0-145.0) #	11.6(4.1-101.0)	8.6(3.6-89.0)
WCC (10 ⁹ /L)	6.5±1.2	7.8±2.3	5.4±1.8	4.8±1.7
CRP (mg/L)	0.56±0.08	0.71±0.13	0.47±0.13	0.24±0.11
Leptin (pg/mL)	3.55±0.54	7.21±1.83*#	7.46±1.74*	3.46±0.31
IL-5 (pg/mL)	6.35±1.12*	9.58±3.61*#	1.57±0.29	1.46±0.37
IL-17 (pg/mL)	124.5±38.9*	176.2±44.5*#	67.5±36.8	56.2±32.4
IFN-γ (pg/mL)	23.4±5.6*	21.6±4.8*	53.2±24.6	59.3±18.2
IL-10 (pg/mL)	12.3±0.29*	8.77±4.58*#	34.2±5.98	28.1±6.25
TGF-beta (pg/mL)	17.2±6.97*	11.5±5.22*#	41.3±13.5	38.2±13.1

a Data presented as median values (minimum-maximum) or mean ± SD..

* compared with control group, p<0.05.

compared with AR without obesity group, p<0.05.

AR, allergic rhinitis. ECP, eosinophil cationic protein, WCC, white cell count. CRP, C reactive protein.

Table 4 Multiple linear regression between leptin and inflammatory cells and markers

Variables	Standardized coefficient (95% CI)	Adjusted R ²	<i>P</i>
Eosinophil (count/mm ³)	0.48 (0.15 to 1.54)	0.65	0.002
Neutrophil (count/mm ³)	0.21 (0.03 to 0.83)	0.01	0.178
Basophil (count/mm ³)	0.37 (0.11 to 1.23)	0.02	0.245
Monocytes (count/mm ³)	0.23 (0.04 to 0.88)	0.03	0.345
Lymphocyte (count/mm ³)	0.19 (0.02 to 0.51)	0.05	0.167
ECP (ng/ml)	0.56 (0.02 to 0.97)	0.49	0.001
WCC(10 ⁹ /L)	0.13 (0.01 to 0.43)	0.36	0.002
CRP (mg/L)	0.51 (0.03 to 1.23)	0.41	0.001
IL-5 (pg/mL)	0.48 (0.05 to 0.91)	0.56	0.003
IL-17 (pg/mL)	0.39 (0.11 to 0.89)	0.67	0.013
IFN- γ (pg/mL)	0.12 (0.01 to 0.45)	0.03	0.153
IL-10 (pg/mL)	-0.43 (-0.02 to 0.65)	0.51	0.001
TGF-beta	-0.65(-0.06 to 1.35)	0.48	0.002

Regression analysis was performed after proper adjustment for age, gender and passive smoke history.

ECP, eosinophil cationic protein, WCC, white cell count. CRP, C reactive protein.

Table 5 Correlation coefficient between leptin and different obesity indicators

	BMI	PBF	WHR	WC
Leptin (Boys)	0.81*	0.69*	0.23	0.75*
Leptin (Girls)	0.73*	0.63*	0.15	0.66*
Leptin (All)	0.74*	0.64*	0.36	0.71*

* p<0.05. BMI, body mass index. PBF, body fat percentage. WC, waist circumference, WHR, waist-to-height.

Table 6 Multiple linear regression between leptin and symptom and medication scores

	Boys			Girls		
	Standardized coefficient (95% CI)	Adjusted R ²	<i>P</i>	Standardized coefficient (95% CI)	Adjusted R ²	<i>P</i>
TNSS score	0.57(0.1 to 1.62)	0.64*	0.003	0.75(0.1 to 1.16)	0.41*	0.001
Runny nose	0.67(0.3 to 1.73)	0.48*	0.004	0.45(0.05 to 0.87)	0.39*	0.003
Sneezing	0.22 (0.01 to 0.7)	0.39*	0.001	0.38(0.02 to 0.9)	0.31*	0.002
Blockage	0.72(0.23 to 1.8)	0.68*	0.003	0.35(0.03 to 1.1)	0.47*	0.001
Itching	0.15(0.01 to 0.41)	0.37*	0.002	0.28(0.05 to 0.9)	0.29*	0.004
VAS score	0.41(0.22 to 1.1)	0.48*	0.001	0.41(0.31 to 1.2)	0.51*	0.005
Medication score	0.69(0.4 to 1.64)	0.41*	0.005	0.55(0.1 to 1.33)	0.33*	0.004

Regression analysis was performed after proper adjustment for age, gender and passive smoke history.

TNSS, total nasal symptom score. VAS, visual analog scale.

Table E1 Correlation between BMI and other obesity indicators according to gender

BMI	PBF	WHR	WC
Boys	$r=0.82, P<0.01$	$r=0.81, P<0.01$	$r=0.76, P<0.01$
Girls	$r=0.87, P<0.01$	$r=0.83, P<0.01$	$r=0.82, P<0.01$
Total	$r=0.84, P<0.01$	$r=0.82, P<0.01$	$r=0.80, P<0.01$