Clinical implications of serum retinol-binding protein 4 in asthmatic children

Yeo Hoon Park

Department of Medicine

The Graduate School, Yonsei University

Clinical implications of serum retinol-binding protein 4 in asthmatic children

Directed by Professor Kyu-Earn Kim

The Master's Thesis submitted to the Department of Medicine,

the Graduate School of Yonsei University

in partial fulfillment of the requirements for the degree of

Master of Medical Science

Yeo Hoon Park

December 2007

This certifies that the Master's Thesis
of Yeo Hoon Park is approved.

Thesis Supervisor : Kyu-Earn Kim

Thesis Committee Member#1 : Myung Hyun Sohn

Thesis Committee Member#2 : Jung Won Park

The Graduate School
Yonsei University

December 2007

ACKNOWLEDGEMENTS

I would like to express my gratitude to all those who gave me the possibility to complete this thesis. I am deeply indebted to my supervisor Prof. Kyu-Earn Kim who gave me the wonderful opportunity to be a Master. And I would also like to thank Prof. Jung Won Park, Myung Hyun Sohn and Dr. Kyung Won Kim whose stimulating suggestions and encouragement helped me in all the time of research and writing of this thesis. I am obliged to Kyung Eun Lee, Ph.D from the institute of allergy for her kind help in experiments.

My fellow residents from the department of pediatrics supported me in my research work. They gave me pleasant memories and power to endure difficulties in studying. I really appreciate them for their help, support and valuable hints.

Especially I would like to give my special thanks to my families for taking care of me patiently and helping me with everything for the completion of this work.

Through the last 2 years, I could have learned not only clinical procedures but also the method of scientific thinking, how to identify a

research problem, how to form and carry out a research plan and how to get results with even attitude to my life. I am sure what I have learned here will greatly benefit my future careers and I am willing to be the one not to be contrary to your expectations.

December 2007

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Abstract

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Aim : Recently the prevalence of both asthma and obesity have increased substantially in many countries. The aim of this study was to evaluate the role of retinol-binding protein 4 (RBP4) in childhood asthma and its association with atopy markers, pulmonary function, and bronchial hyperresponsiveness in relation to obesity.

Methods : We studied 160 children aged 6 to 10 years old, including 122 asthmatics and 38 controls. The body mass index, pulmonary function tests, and methacholine challenge tests were measured on the same day. Total eosinophil count, total serum IgE, serum eosinophil cationic protein, and serum RBP4 were measured in all subjects.

Results : There was no difference in serum RBP4 levels between asthmatics and the control group. In all subjects or subgroups, serum RBP4 did not associate with total eosinophil count, total serum IgE, serum eosinophil cationic protein, or PC_{20}. There was no relationship between serum RBP4 and pulmonary function in female subjects. But, FEV_1/FVC and $FEF_{25-75\%}$ contributed to serum RBP4 in male subjects.

Conclusion : Our findings show an association between RBP4 and pulmonary function in prepubertal males. This relationship may indirectly affect the high prevalence of childhood asthma in males.

Key words : asthma, children, pulmonary function, retinol-binding protein 4

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I. INTRODUCTION

Asthma is one of the most common chronic illnesses in children, and obesity is another important public health problem that can affect children.[1, 2] Recently the prevalence of both asthma and obesity have increased substantially in many countries, leading to speculation that obese people might be at an increased risk of developing asthma.[3, 4] Although a large number of studies have been performed, the possibility that obesity might increase the risk of asthma development remains controversial.[5] Nevertheless, potential mechanisms have been suggested that obesity-related changes in lung volumes, systemic inflammation, and other adipocyte-derived factors such as leptin, adiponectin, resistin, and visfatin, as well as cytokines and chemokines that might alter smooth muscle function in such a way as to promote airway narrowing.[2, 6]

The obese state is characterized by low-grade systemic inflammation, as indicated by increased levels of the inflammatory markers CRP and IL-6 in the circulation of obese subjects.[6] The effects of increased BMI on asthma may be mediated by upregulation of inflammatory mechanisms in the airway epithelium. [7] The current view of adipose tissue is that of an active

secretory organ, sending out and responding to signals that modulate appetite, energy expenditure, insulin sensitivity, endocrine and reproductive systems, bone metabolism, and inflammation and immunity.[6] The serum concentrations of many adipokines could impact airway function, which might lead to asthma in both human and murine subjects with obesity.[8] A recent study showed that leptin, an adipokine, might play a role in atopic asthma in children.[7] Retinol-binding protein 4 (RBP4) is highly expressed in isolated mature human adipocytes and secreted by differentiating human adipocytes.[9] Elevated serum RBP4 levels are associated with diabetes and the components of the metabolic syndrome, including body-mass index, waist-to-hip ratio, serum triglyceride levels, systolic blood pressure and decreased levels of high-density lipoprotein cholesterol.[10]

There are no reports on the relationship between RBP4 and childhood asthma. We evaluated the role of RBP4 in childhood asthma and its association with atopy markers, pulmonary function, and bronchial hyperresponsiveness.

II. MATERIALS AND METHODS

1. Subjects

One hundred sixty subjects, 122 asthmatics, 38 controls were recruited from Severance Hospital, Yonsei University College of Medicine. Athma was defined as recurrent wheezing or coughing episodes in the absence of a cold in the preceding 12 months. Additionally a physician's diagnosis of bronchial hyperresponsiveness upon methacholine challenge ($PC_{20} \leq$ 16 mg/mL) and at least 12% reversibility of forced expiratory volume in 1 second (FEV_1) after inhaling a β_2 agonist, as per criteria of the American Thoracic Society (ATS), were required.[11] All participants were enrolled before administering oral or inhaled corticosteroids. Children with a history of asthma attacks requiring systemic corticosteroids within the preceding 6 months were excluded. The control participants were age-matched healthy children who visited the hospital for a general health work-up or vaccination and had no history of wheezing, recurrent chronic disease, infection during the preceding two weeks, or hypersensitivity to methacholine.

Height and weight were measured in light clothing without shoes to calculate BMI in kg/m^2. Obesity and overweight classification were defined by BMI for age and sex based on an international survey.[12] Absolute BMI values were then converted to previously established BMI percentiles of Korean children for data analysis.[13] The 85 th and 95 th percentiles of BMI for age and sex were used as cut off points to identify normoweight, overweight and obesity. Informed written consent was obtained from all participants before inclusion in the study. The study was approved by the Severance Hospital Institutional Review Board beforehand.

2. Spirometry and methacholine challenge test

Lung function was measured by spirometry (V_{max} encore ; VIASYS Healthcare Inc., Conshohocken, USA) according to ATS standards.[11] Bronchial hyperresponsiveness was assessed in all participants by the methacholine challenge test. Children were eligible if they could perform reproducible spirometry and had an FEV_1 of at least 70% of the predicted value.[14] In short, after saline inhalation, doses of methacholine (0.075, 0.15, 0.31, 0.62, 1.25, 2.5, 5, 10.25 mg/mL) were delivered for 0.6 seconds through a DeVilbiss 646 nebulizer using a Rosenthal-French dosimeter (Ferraris, Hertford, England) until either FEV_1 decreased by 20% or more, or the highest dose administered. FEV_1 was measured 60 to 90 sec after each inhalation. The methacholine concentration inducing a 20% decrease in FEV_1 (PC_{20}) was recorded. The challenge test was considered positive if the PC_{20} was 16 mg/mL or less. Anti-inflammatory preparations and bronchodilators were withheld for 24 hours before the test.

3. Measurement of blood eosinophils, total serum IgE, serum eosinophil cationic protein (ECP) and serum RBP4 levels

The NE-8000 system (Sysmex, Kobe, Japan) was used to count eosinophils automatically in peripheral blood. Total serum IgE and ECP were measured by the CAP radioallergosorbent technique (UniCAP ; Pharmacia and Upjohn, Uppsala, Sweden). Blood samples to measure serum RBP4 levels were obtained between 6 AM and 9 AM after an overnight fast. After clotting at 4℃, the serum was separated by centrifugation at 1,300 g for 10 min and stored at -70℃ refrigerator until the assays. Serum RBP4 concentrations were measured with a human RBP4 Competitive ELISA Kit (AdipoGen, Seoul, Korea) according to the manufacturer's instructions. The minimum RBP4 detection limit was 1 ng/mL and all assays were performed in duplicate for each sample, with the mean values reported here.

4. Statistical analysis

Descriptive statistics were calculated as the mean±SD or as median (interquartile range) for continuous variables. Comparisons between groups were made using a Student's t-test or Mann-Whitney U test. Simple linear regression analysis was performed to assess the BMI, FEV_1, FEV_1/FVC, $FEF_{25-75\%}$, PC_{20}, eosinophil count, total serum IgE and ECP on RBP4 levels. A *P*-value less than .05 was considered statistically significant. All analyses were performed on the Statistical Package for the Social Sciences software (version 13.0, SPSS Inc, Chicago, IL, USA).

III. RESULTS

The participants consisted of 102 males (63.8%) and 58 females (36.2%) (mean age, 8.6 ± 1.2 years). The mean values for age and BMI percentile were not different between the asthmatic and healthy children. Males were more predominant in the asthmatic group. Eosinophil count, total serum IgE and ECP were statistically higher in asthmatic than control subjects. $FEF_{25-75\%}$ was significantly lower in asthmatic than control subjects (Table 1).

Table 1 Selected characteristics to asthma status of participants

Characteristics	Asthma (n=122)	Control (n=38)	*P*
Age (years)*	8.6±1.2	8.4±0.9	.115
Male/Female	84/38	18/20	
BMI percentile*	64.66±30.33	63.89±30.43	.893
Normoweight/overweight/obesity (%)	78/22/22	23/6/9	
	(63.9/18.0/18.0)	(60.5/15.8/23.7)	
Eosinophil count (/μL)†	305.00	150.00	.011
	(190.00-700.00)	(70.00-232.50)	
Serum total IgE (U/mL)†	206.50	45.80	.000
	(79.73-581.50)	(31.23-87.50)	
Serum ECP (μg/L)†	14.60 (5.91-36.00)	7.39 (3.89-11.48)	.000
FEV_1/FVC (% predicted)*	102.91±8.25	105.28±4.76	.099
Baseline FEV_1(% predicted)*	83.46±14.80	91.66±12.85	.058
$FEF_{25-75\%}$ (% predicted)*	79.70±23.55	90.81±26.34	.024

*mean±SD, †median (interquartile range)

The mean RBP4 concentration, shown in Fig. 1, was not significantly different between the asthma and control subjects (mean±SD 256.91±93.37 μg/mL versus 264.20±151.79 μg/mL, *P* = NS).

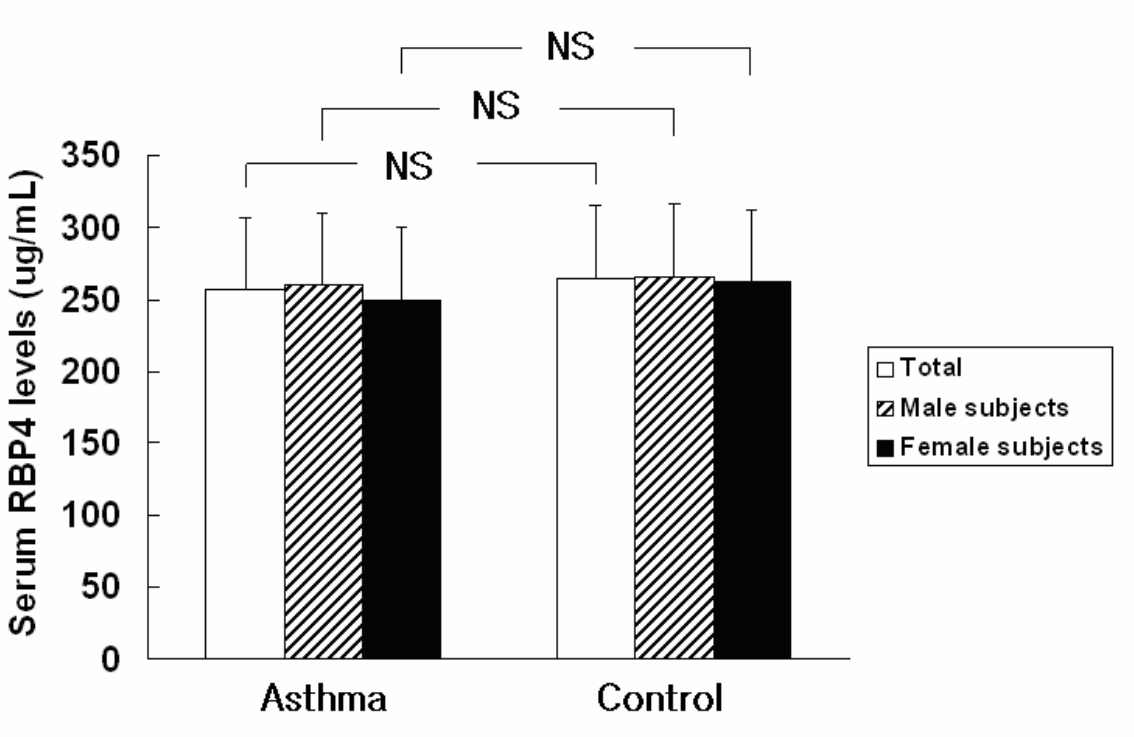

Fig. 1. Serum RBP4 levels between the asthmatic and the control subjects. Serum RBP4 levels did not show any difference between the asthmatics and the controls in total, male and female subjects. (*P*>0.05) Data represent median values.

Simple linear regression analysis was performed with RBP4 as the dependent variable and age, BMI percentile, eosinophil count, total serum IgE, ECP, FEV_1, FEV_1/FVC, $FEF_{25-75\%}$ and PC_{20} as the independent variables for subjects divided by sex (Table 2). The results indicate that

FEV_1/FVC and $FEF_{25-75\%}$ contribute significantly to serum RBP4 level in males. Only $FEF_{25-75\%}$ contributed to serum RBP4 level in males on multiple linear regression analysis (data not shown). No other parameters were shown to contribute to RBP4. No variables significantly contributed to RBP4 in females. In normoweight, overweight and obesity group, no variables contributed to RBP4 in both sex (data not shown).

Table 2 Simple linear regression analysis between RBP4 and variables in all participants

Variables	Total (n=160)							
	Male (n=102)				Female (n=58)			
	Unstandardized coefficients		Standardized coefficients		Unstandardized coefficients		Standardized coefficients	
	B	SE	Beta	*P*	B	SE	Beta	*P*
BMI	0.002	0.003	0.081	0.417	0.007	0.007	0.146	0.307
TEC	0.000	0.000	0.060	0.546	0.000	0.000	0.019	0.897
IgE	0.000	0.000	0.164	0.100	0.001	0.000	0.185	0.195
PC20	-0.003	0.005	-0.071	0.481	0.004	0.009	0.058	0.687
ECP	-0.002	0.003	-0.057	0.578	0.011	0.010	0.154	0.291
FEV_1	-0.010	0.006	-0.187	0.081	0.004	0.022	0.026	0.867
FVC	-0.007	0.006	-0.123	0.255	0.016	0.022	0.117	0.461
FEV_1/FVC	-0.026	0.010	-0.275	0.016	-0.021	0.047	-0.080	0.667
$FEF_{25-75\%}$	-0.010	0.003	-0.313	0.003	0.001	0.010	0.022	0.887

In all analyses, the dependent variable was RBP4

B : Unstandardized regression coefficient

SE : Standard error

Beta : Standardized regression coefficient

P : P value

Abbreviations : BMI : body mass index, TEC : total eosinophil count, ECP : eosinophil cationic protein

IV. DISCUSSION

The aim of this study was to investigate serum RBP4 levels and its relationship between the parameters of atopy, pulmonary function, and bronchial hyperresponsiveness. There were no differences in mean serum RBP4 concentrations between the asthmatics and the controls. There was also no significant correlation between serum RBP4 and BMI for both the asthmatic and control group. But, FEV_1/FVC and $FEF_{25-75\%}$ contributed to serum RBP4 levels in male participants.

There are several proposed mechanisms by which obesity can affect airway function.[15] A recent review touched on the mechanical, immunological, hormonal and inflammatory effects of obesity that may play a role in the development and persistence of asthma.[15, 16] Although a large number of cross-sectional, case-control, prospective and weight loss studies have been performed, the possibility that obesity might increase the risk of asthma development remains controversial.[3, 15] Our findings suggest that there is no direct association between BMI and asthma. In normoweight, overweight and obesity group, there were no relationships between RBP4 and pulmonary functions, atopy markers and airway hypersensitiveness. Although a small study sample and a relatively small obese group may have affected these results, the proportion of obese people in our study is similar to the proportion of obese people in the general Korean population. Both the low proportion of obesity and the rarity of severe obesity in Korea may also have affected the results. The fact that the subjects were prepubertal (younger than 10) may also have influenced the results by excluding the effects of sex hormones. Although the possibility that obesity might increase the risk of asthma development remains controversial, the correlation between them is still a matter of concern. It is well documented that obesity is an inflammatory state that leads to increased levels of hormones such as leptin, cytokines such as

interleukin-6 and tumor necrosis factor-α, chemokines and other inflammatory mediators that could potentially influence airway smooth muscle.[6] RBP4 is a newly discovered fat-derived peptide that modulates glucose metabolism and consequently induces insulin resistance.[17] A recent report suggests that RBP4 is a central mediator of obesity-induced insulin resistance in mice and humans.[18] RBP4 was selectively elevated in adipose GLUT4 knockout mice and obese humans with type 2 diabetes mellitus, suggesting that RBP4 may contribute to the pathogenesis of insulin resistance in diabetes.[18] Mean serum RBP4 levels were elevated in both nondiabetic and diabetic obese subjects, and serum RBP4 levels were positively correlated with BMI.[19] Previous studies have shown that RBP4 levels are elevated in obese patients.[18-20] On the other hand, another report showed no association between plasma RBP4 levels and BMI.[21] Our results show that serum RBP4 is not related to BMI. RBP4 was not different between the asthma and normal control groups, and there were no differences in BMI between the asthmatics and controls in our study. The relatively narrow BMI distribution of our study subjects might explain the lack of a relationship.

Simple linear regression analysis revealed that both FEV_1/FVC and $FEF_{25-75\%}$ are predictive factors of RBP4 for male participants in our study. The FEV_1 to FVC ratio is a useful assessment of airflow limitation.[22] Recently, FEV_1/FVC was reported to be a predictive factor for basement membrane thickness, which is a known early airway remodeling marker.[23] Among the respiratory indices derived from the forced vital capacity maneuver, maximal midexpiratory flow rate (MMEF) namely forced expiratory flow between 25% and 75% of the lung volume ($FEF_{25-75\%}$), which measures the average flow of gas through the middle lung volumes, was originally considered a more sensitive and earlier marker of obstruction in the small airways than FEV_1.[24, 25] Our findings show that these two parameters contribute to serum RBP4 levels. The relationship between serum RBP4 and pulmonary function might be indirectly associated

with lung inflammation, changes in small airways, and airflow obstruction, all part of asthma pathogenesis. It is known that asthma is more prevalent in males younger than 10 years or the mid-teens, at which point asthma is more prevalent in females until sexual maturity.[26] A current study shows that high serum leptin levels in asthmatic boys may partly explain the higher prevalence of childhood asthma in male.[7] Our results support this phenomenon. No explanation for the phenomenon has been given. The often hypothesized role of sexual hormones still remains to be demonstrated.[26]

Our research establishes the first causal relationship study between serum RBP4 and asthma. We excluded the effects of sex hormones by recruiting prepubertal children as study subjects. The small number of participants and the relatively small obese group are limitations of this study.

V. CONCLUSION

We evaluated the role of retinol-binding protein (RBP) 4 in childhood asthma by recruiting one hundred sixty prepubertal children. Our findings show an association between RBP4 and pulmonary function in prepubertal males. This relationship may indirectly affect the high prevalence of childhood asthma in male. Additional studies between adipokines and asthma will be helpful to investigate the pathogenesis of asthma associated with obesity.

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Abstract (in Korean)

소아 천식 환자에서

혈청 Retinol-Binding Protein 4의 임상적 의미

<지도교수 김 규 언>

연세대학교 대학원 의학과

박 여 훈

목적 : 최근 천식과 비만의 유병률이 증가하고 있어 천식과 비만 사이의 연관성을 밝히고자 하는 연구가 지속되고 있다. Retinol-binding protein (RBP) 4는 지방세포(adipocyte)에서 분비되는 물질로서 최근 비만 및 당뇨병과 밀접한 연관이 있는 것으로 알려지고 있다. 본 연구에서는 비만과 관련된 천식의 병인에 관한 연구의 일환으로 소아 천식에서 RBP4의 의의를 규명하고자 하였으며, 아토피 인자, 폐기능 지표 및 기관지 과민성과의 연관성에 대해 알아보고자 하였다.

대상 및 방법 : 만 6세에서 10세 사이의 160명을 대상으로 하였다. 천식 환아는 122명, 대조군은 38명이었다. 전체 대상아에서 폐기능 검사, 메타콜린 유발시험을 시행하였고, 혈액 총호산구수, 혈청 총 IgE, eosinophil cationic protein(ECP), RBP4 농도 및 키와 몸무게를 측정하였다. Body mass index (BMI)를 구하여 각 성별, 연령에 따른 BMI 백분위수 곡선을 이용하여 BMI 백분위수를 구하였다.

결과 : 전체 대상아에서 혈청 RBP4 농도는 천식군과 대조군 사이에

의미있는 차이를 보이지 않았으며 두 군 모두에서 BMI와 관련이 없었다. 전체 대상아와 여아에서 혈청 RBP4 농도는 혈액 총호산구수, 혈청 총 IgE, ECP 농도, 폐기능 및 PC_{20}와 연관성을 나타내지 않았으나 남아에서 혈청 RBP4 농도는 FEV_1/FVC 및 $FEF_{25\%-75\%}$와 의미있는 상관관계를 보였다.

결론 : 혈청 RBP4는 천식과 직접적인 관련은 보이지 않았으나, 사춘기 이전 남아에서 폐기능과 연관성을 나타내었다. 이는 사춘기 이전 소아에서의 천식 유병률이 남아에서 증가되어 있는 경향을 간접적으로 뒷받침하는 것으로 사료된다.

핵심되는 말 : 천식, 소아, 폐기능, Retinol-binding protein 4