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Association of serum retinol binding protein 4 and insulin resistance in apparently healthy adolescents

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Abstract

Insulin resistance constitutes a pathophysiologic link between obesity, atherosclerosis, and/or cardiovascular complications. Retinol binding protein 4 (RBP4) is a newly discovered adipocyte product that modulates glucose metabolism and consequently induces insulin resistance. We investigated the association between serum RBP4 levels and insulin resistance in obese and nonobese adolescents. A total of 87 nonobese (60 males and 27 females) and 85 obese (62 males and 23 females) apparently healthy adolescents, 12 to 18 years old, were included in this study. A questionnaire was used to obtain participant medical history and lifestyle information, such as smoking and alcohol ingestion habits. Subjects' anthropometric measurements were taken to calculate for body mass index and waist-to-hip ratio. Serum RBP4 levels were measured by an enzyme immunoassay kit. High-sensitivity C-reactive protein, fasting glucose, total cholesterol, triglycerides, high-density lipoprotein cholesterol, and fasting insulin were measured. Low-density lipoprotein cholesterol level and homeostatic model assessment of insulin resistance (HOMA-IR) were calculated. Males had significantly higher RBP4 levels than females. Serum RBP4 levels were significantly higher in the obese group compared with the nonobese group. In all subjects, RBP4 was positively correlated with adiposity index (body mass index, waist circumference, waist-to-hip ratio), systolic and diastolic blood pressures, glucose tolerance index (fasting glucose, insulin, HOMA-IR), lipid profile (total cholesterol, triglycerides), and inflammatory indices (high-sensitivity C-reactive protein, white blood cell count). In multiple linear regression analysis, RBP4 was independently associated with age, HOMA-IR, and triglyceride levels in the nonobese group and with sex and triglyceride levels in the obese group. These results suggest that serum RBP4 might have clinical implications for lipid metabolism and insulin action in adolescents.

1. Introduction

The association of insulin resistance with an increased risk of cardiovascular disease (CVD) is well known [1] and constitutes a pathophysiologic link between obesity, atherosclerosis, and/or cardiovascular complications [2-4].

Retinol binding protein 4 (RBP4) is a newly discovered fat-derived peptide that modulates glucose metabolism and consequently induces insulin resistance [5]. A recent report by Yang et al [6] suggests that RBP4 is a central mediator of obesity-induced insulin resistance in mice and humans. The discovery of RBP4 provides a new link between obesity and insulin resistance. Given that the prevalence of obesity has been increasing rapidly [7] and that risk factors for CVD may initiate the process of atherosclerosis during childhood [8], the role of RBP4 as a mediator for increased insulin resistance in young individuals has clinical implications.

At the same time, clinical implications of RBP4 levels in obese humans are far from clear. The number of such studies on humans [6,9] is relatively small, making it difficult to draw conclusions. Moreover, to our knowledge, no other study of the general population, especially of adolescent subjects, has been published.

Accordingly, we investigated the association between serum RBP4 levels and insulin resistance in obese and nonobese adolescents.

2. Subjects and methods

2.1. Subjects

All subjects signed an informed consent form approved by the hospital's ethical committee. School-based volunteers

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were recruited by public advertisement written by the educational institution. A total of 87 nonobese (60 males and 27 females) and 85 obese (62 males and 23 females) apparently healthy adolescents, aged 12 to 18 years, were included. Subjects were excluded if they had a medical history of, or upon physical examination evidence of, CVD, diabetes, hypertension (resting blood pressure [BP] >140/90 mm Hg), a body weight fluctuation of more than 5 kg in the previous 6 months, endocrine disorders, or medication that could affect cardiovascular function or metabolism. We used a questionnaire to assess past and/or current medical illnesses of the study subjects, as well as their lifestyle choices, such as alcohol ingestion and cigarette smoking. Alcohol ingestion was defined as consuming alcohol more than once for each week. Smoking was defined as current cigarette smoking.

2.2. Methods

Anthropometric measurements were taken from lightly clothed subjects without shoes. Height to the nearest 0.1 cm and weight to the nearest 0.1 kg were measured by an automatic height-weight scale, and body mass index (BMI) was calculated as the weight in kilograms divided by the square of height in meters. Waist circumference was measured at the midpoint between the lower border of the rib cage and the iliac crest, hip circumference was measured at the widest part of the hip region, and thigh circumference was measured 10 cm proximal to the superior patella border. To reduce variation in measurements, one person took all anthropometric measurements throughout the study.

Blood samples were obtained from each subject after an 8-hour overnight fast by venipuncture into plain and EDTA tubes. Serum RBP4 levels were measured with an enzyme immunoassay kit (AdipoGen, Seoul, Korea), and inter- and intra-assay variability were 7.2% and 5.5%, respectively. High-sensitivity C-reactive protein (hs-CRP), fasting glucose, total cholesterol, triglycerides, and high-density lipoprotein cholesterol (HDL-C) levels were measured with an ADVIA 1650 Chemistry system (Bayer, Tarrytown, NY). Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation [10]. Fasting insulin was measured by electrochemiluminescence immunoassay (Roche, Indianapolis, IN). Insulin resistance was estimated by the homeostatic model assessment of insulin resistance (HOMA-IR), with calculations as follows: HOMA-IR = [fasting insulin (μIU/mL) × fasting glucose (mg/dL)/18]/22.5. White blood cell (WBC) counts were assayed using an ADVIA 2120 Hematology system (Bayer).

2.3. Statistical analysis

Data are expressed as mean ± SD. Variables such as RBP4, hs-CRP, triglycerides, and HOMA-IR levels were logarithmically transformed before statistical analysis to approximate normal distributions. However, mean values of the variables are presented with untransformed data to aid in interpretation. Clinical and metabolic characteristics between sex and obesity were compared using a t test for continuous variables and either the χ^2 test or the Fisher exact test for categorical variables. Pearson correlation coefficients were calculated to evaluate the relationship between RBP4 levels and clinical and metabolic variables. A multiple linear regression analysis was performed to determine the association between RBP4 levels (as a dependent variable) and the explanatory variables of age, sex, HOMA-IR, systolic BP, diastolic BP, waist circumference, BMI, total cholesterol, HDL-C, triglycerides, hs-CRP, a history of smoking (no = 0, yes = 1), and alcohol ingestion (no = 0, yes = 1). Data from 86 nonobese and 78 obese adolescents who completed the lifestyle questionnaire were used in the multivariate analysis. Results were considered significant when $P < .05$. All calculations were performed using SAS 8.01 (SAS institute, Cary, NC).

Table 1
Clinical and metabolic variables of nonobese and obese groups

Variables	Nonobese (n = 87)	Obese (n = 85)	P
Age (y)	14.5 ± 1.3	14.8 ± 1.3	.07
Sex			
Male	60	62	.57
Female	27	23	
Adiposity index			
BMI (kg/m^2)	19.6 ± 1.9	30.8 ± 2.3	<.01
Waist (cm)	67.9 ± 7.0	95.1 ± 7.9	<.01
WHR	0.77 ± 0.06	0.88 ± 0.05	<.01
BP (mm Hg)			
Systolic	111.5 ± 8.8	131.6 ± 11.2	<.01
Diastolic	58.2 ± 5.1	67.5 ± 7.6	<.01
Glucose tolerance index			
Fasting glucose (mg/dL)	81.0 ± 8.2	83.4 ± 9.5	.08
Fasting insulin (μIU/mL)	8.5 ± 5.9	22.0 ± 16.3	<.01
HOMA-IR	1.7 ± 1.3	4.7 ± 3.9	<.01
RBP4 (μg/mL)	26.7 ± 7.0	32.8 ± 9.2	<.01
Lipid profile			
Total cholesterol (mg/dL)	153.4 ± 23.1	180.5 ± 30.7	<.01
Triglyceride (mg/dL)	72.3 ± 32.2	141.6 ± 91.3	<.01
HDL-C (mg/dL)	49.1 ± 8.3	42.5 ± 7.9	<.01
LDL-C (mg/dL)	89.8 ± 20.5	109.7 ± 29.0	<.01
Inflammatory index			
Hs-CRP (mg/mL)	0.1 ± 0.34	0.17 ± 0.27	<.01
WBC ($10^3/\mu$L)	6.5 ± 1.6	7.7 ± 1.5	<.01
Lifestyle			
Smoking[a] (n)	6 (87)	4 (79)	.75[b]
Alcohol[c] (n)	1 (86)	9 (78)	<.01

Data are shown as mean ± SD and number. Retinol binding protein 4, hs-CRP, triglyceride, and HOMA-IR levels were logarithmically transformed before statistical analysis to approximate normal distribution; however, untransformed data are shown in the table. P values were calculated by t test and χ^2 text.

[a] Number of adolescents reporting active smoking at present. (A total of 87 nonobese and 79 obese adolescents completed the questionnaire for smoking habit).

[b] In cells with an expected count of less than 5, P was calculated by Fisher exact test.

[c] Number of adolescents reporting alcohol ingestion at least 1 time a week. (A total of 86 nonobese and 78 obese adolescents completed to the questionnaire for the habit of alcohol ingestion).

3. Results

3.1. RBP4 levels in the nonobese group and the obese group

Mean values of serum RBP4 were 32.17 ± 9.05 μg/mL in males (n = 122) and 26.14 ± 6.46 μg/mL in females (n = 50), and significantly higher in male adolescents ($P < .01$). The clinical characteristics of nonobese and obese adolescents are shown in Table 1. There was no significant difference in mean age, sex, fasting glucose level, or smoking habits between the 2 groups. The obese group had a significantly higher BMI, waist circumference, waist-to-hip ratio (WHR), systolic BP, diastolic BP, fasting insulin, HOMA-IR, total cholesterol, triglycerides, LDL-C, hs-CRP, WBC levels, and alcohol ingestion than the nonobese group ($P < .01$ for all). In addition, RBP4 levels were significantly higher in the obese group than in the nonobese group ($P < .01$). In contrast, HDL-C levels were significantly lower in the obese group than in the nonobese group ($P < .01$).

3.2. Correlation between RBP4 levels and insulin resistance and other parameters

RBP4 levels were positively correlated with adiposity indices, systolic and diastolic BPs, fasting glucose and insulin, HOMA-IR, total cholesterol, triglycerides, and inflammatory indices such as hs-CRP and WBC count, as shown in Table 2. However, after subgroup analysis, most of these associations disappeared, especially in the obese group. In the nonobese group, RBP4 levels were positively correlated with systolic BP ($r = 0.31$, $P < .01$), diastolic BP ($r = 0.23$, $P < .05$), fasting glucose ($r = 0.22$, $P < .05$), HOMA-IR ($r = 0.30$, $P < .01$), and triglyceride levels ($r = 0.24$, $P < .05$). In the obese group, RBP4 levels were positively correlated with triglyceride levels ($r = 0.28$, $P < .01$), but nonsignificantly correlated with HOMA-IR ($r = 0.16$, $P = .14$) (Fig. 1).

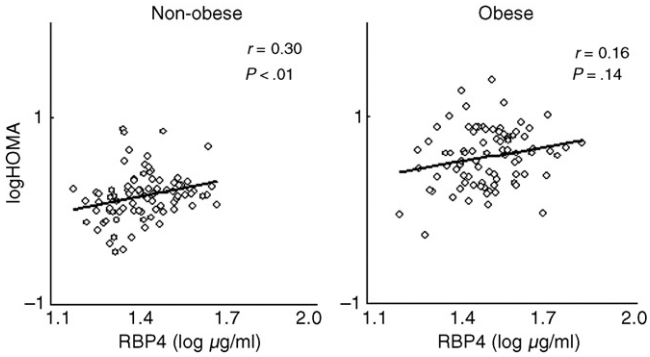

Fig. 1. The relationship between serum RBP4 levels and HOMA-IR in the nonobese and obese groups. RBP4 levels were positively correlated with HOMA-IR in the nonobese group (n = 87) ($r = 0.30$, $P < .01$). However, a significant correlation was not present in the obese group (n = 85) ($r = 0.16$, $P = .14$).

3.3. Multiple linear regression assessment for independent relationships between RBP4 and clinical variables

In the multiple linear regression analysis, RBP4 was found to be independently associated with age, HOMA-IR, and triglyceride levels in the nonobese group and independently associated with sex and triglyceride levels in the obese group, as shown in Table 3.

Table 2
Correlations between RBP4 levels and various parameters (N = 172)

	RBP4	
	r	*P*
Age (y)	−0.06	.41
Adiposity index		
BMI (kg/m^2)	0.31	<.001
Waist (cm)	0.32	<.001
WHR	0.20	<.01
BP (mm Hg)		
Systolic	0.40	<.001
Diastolic	0.33	<.001
Glucose tolerance index		
Fasting glucose (mg/dL)	0.20	<.01
Fasting insulin (μIU/mL)	0.26	<.001
HOMA-IR	0.36	<.001
Lipid profile		
Total cholesterol (mg/dL)	0.26	<.001
Log triglyceride (mg/dL)	0.41	<.001
HDL-C (mg/dL)	−0.12	.11
LDL-C (mg/dL)	0.14	.06
Inflammatory index		
hs-CRP (mg/mL)	0.17	<.05
WBC (10^3/μl)	0.19	<.05

Coefficients (*r*) and *P* values were calculated by the Pearson correlation analysis. Retinol binding protein 4, hs-CRP, triglyceride, and HOMA-IR levels were logarithmically transformed before statistical analysis to approximate normal distribution.

Table 3
Multiple regression analysis to assess independent relationships between RBP4 and clinical variables

Variables	Parameter estimate	SE	*P*
Nonobese[a]			
Age	-0.05	0.02	<.05
HOMA-IR	0.07	0.03	<.05
Triglyceride	0.23	0.73	<.01
Obese[b]			
Sex[c]	0.19	0.18	.07
Triglyceride	0.08	<0.01	<.05

Variables significantly associated with RBP4 ($P < .05$) are shown. Retinol binding protein 4, triglyceride, hs-CRP, and HOMA-IR levels were logarithmically transformed before statistical analysis to approximate normal distribution.

[a] Data from a total of 86 nonobese adolescents who completed the lifestyle questionnaire were used in a multiple linear regression model, which included age, sex, HOMA-IR, systolic BP, BMI, total cholesterol, HDL-C, triglyceride, hs-CRP, and a history of smoking and alcohol ingestion as independent variables ($R^2 = 0.32$; F value, 3.14; $P < .01$).

[b] Data from a total of 78 obese adolescents who completed the lifestyle questionnaire were used in a multiple linear regression model with the same independent variable as the nonobese group ($R^2 = 0.28$; F value, 2.32; $P < .05$).

[c] Male, 0; female, 1.

4. Discussion

RBP4, a peptide secreted from adipocytes in addition to hepatocytes, provides a new link between obesity and insulin resistance [5]. Expression of glucose transporter 4 (GLUT4) is greatly reduced in adipose tissue with the development of insulin resistance [1]. Recent research has shown that 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 [6]. Although RBP4 has been associated with insulin resistance, the implications of this relationship in the general population of humans, especially adolescents, has not yet been established. Traditional risk factors for CVD may initiate the atherosclerosis process during childhood [11,12], making it worthwhile to determine whether serum RBP4 in obese adolescents affects insulin resistance or not.

In this study, we confirmed that serum RBP4 levels were elevated in obese adolescents, which is consistent with previous studies [6,9]. In addition, we found that serum RBP4 levels were significantly associated with insulin resistance measured as HOMA-IR in nonobese adolescents, even after adjusting for potential covariates that are known to be linked to insulin resistance, such as CVD risk factors and/or components of metabolic syndrome. However, this association was no longer significant in obese adolescents. The reason serum RBP4 was a more powerful determinant of insulin resistance in nonobese than in obese adolescents is not clear, but several possible explanations could be considered for this discrepancy. First, since the discovery of leptin [13], many fat-derived hormones that regulate glucose homeostasis have been identified. Diverse factors, including RBP4, may affect insulin resistance in obese adolescents. Second, there may be a type II error in the analysis of the associations between RBP4 levels and HOMA-IR in the obese group. Finally, because the gold standard for the measurements of insulin resistance is known to be a euglycemic hyperinsulinemic clamp [14], HOMA-IR may not reflect the insulin resistance accurately. RBP4 is believed to affect insulin sensitivity by down-regulating the activities of phosphoinositol-3 kinase and the phosphorylation of insulin in muscle, which are key steps in glucose metabolism [5]. Moreover, RBP4 increases hepatic glucose production by up-regulating the expression of phosphoenolpyruvate carboxykinase [5,15].

In this study, serum levels of RBP4 showed significantly positive associations with traditional CVD risk factors and/or components of metabolic syndrome, including adiposity index (BMI, waist circumference, WHR), BP, lipid profile (total cholesterol, triglycerides), and inflammatory markers (hs-CRP, WBC count), in accordance with a previous study [9]. These results suggest that RBP4 might be used as a marker for CVD risk. Multiple regression analysis revealed, however, that most variables except for HOMA-IR and triglyceride levels no longer have a statistically significant association with RBP4. Further studies are needed to explore the clinical implications of RBP4 as a surrogating marker for CVD risk.

Serum RBP4 levels were independently associated with the level of triglycerides in both obese and nonobese adolescents in multiple regression analyses, suggesting that RBP4 may affect fatty acid metabolism. Considering that dysregulation of fatty acid metabolism is closely related with insulin resistance [16], RBP4 might affect insulin action via a dysregulated lipid metabolism, although the pathway is not clear.

In this study, male adolescents had higher RBP4 levels than females, and multiple regression analysis showed that sex was a significant predictor of RBP4 levels in obese adolescents. These results suggest that sex-specific differences in RBP4 concentration may exist, as shown with serum adiponectin [17,18] levels, although the mechanism is not clear.

Given that alcohol ingestion has been known to affect insulin sensitivity and lipid metabolism [19,20], a higher percentage of alcohol ingestion in obese adolescents compared with nonobese adolescents might be a source of bias in this study. However, because the study population consisted of young healthy adolescents, and the percentage of alcohol use was much lower than in the general population [21], the confounding effect of alcohol consumption was minimized.

It should be noted that the cross-sectional design of this study limits the interpretation of the results, especially with regard to cause-effect interactions. Furthermore, we could not evaluate possible interactions between adipokines affecting insulin sensitivity in this study.

In conclusion, we found that serum RBP4 levels showed independent associations with triglyceride levels, and in nonobese adolescents, insulin resistance also had an independent positive association with serum RBP4 level. These results suggest that serum RBP4 might have clinical implications for lipid metabolism and insulin action in healthy adolescents.

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