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Elevated Retinol-Binding Protein 4 Levels Are Associated with Metabolic Syndrome in Chinese People

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Context: High retinol-binding protein 4 (RBP4) is thought to be associated with insulin resistance in humans. However, evidence from large-scale populations about the relationship between RBP4 and metabolic diseases is scarce.

Objective: We evaluated plasma RBP4 distribution and its association with metabolic syndrome (MetS) among middle-aged and older Chinese.

Research Design and Methods: We evaluated plasma RBP4 in a cross-sectional sample of 3289 Chinese aged from 50 to 70 yr in Beijing and Shanghai by using an in-house developed and validated sandwich ELISA. MetS was defined according to the updated National Cholesterol Education Program Adult Treatment Panel III criteria for Asian-Americans.

Results: RBP4 levels were higher in male and Beijing residents, compared with female and Shanghai participants (both $P < 0.001$). RBP4 levels were associated positively with body mass index, waist circumference, triglycerides, total and low-density lipoprotein cholesterol, blood pressure, fasting insulin, and homeostatic model assessment of insulin resistance and negatively with high-density lipoprotein cholesterol and adiponectin (all $P < 0.001$). In the highest RBP4 quartile, the MetS risk was significantly higher (odds ratio 2.58; 95% confidence interval 2.08–3.20) than in the lowest quartile after adjustment for potential confounders. This association remained strong (odds ratio 2.25; 95% confidence interval 1.72–2.94) after further controlling for C-reactive protein, adiponectin, homeostatic model assessment of insulin resistance, and body mass index.

Conclusions: This first large-scale population study shows that elevated RBP4 levels are strongly and independently associated with MetS. Prospective studies are needed to establish the role of RBP4 in the development of MetS and related diseases. **(*J Clin Endocrinol Metab* 92: 4827–4834, 2007)**

INSULIN RESISTANCE IS a hallmark of metabolic syndrome (MetS), a constellation of metabolic abnormalities including central obesity, dyslipidemia, elevated blood pressure, and hyperglycemia (1). MetS is associated with an increased risk of type 2 diabetes mellitus and cardiovascular disease (CVD) (2, 3). The combination of excess caloric intake and a sedentary lifestyle have made obesity and MetS a global epidemic in not only Western countries (4) but also Asian societies (5). Thus, MetS has been considered one of the major public health challenges (4–6). Although insulin resistance is associated with most components of MetS and is believed to play a causal role in its pathogenesis (6, 7), other factors may also contribute to the development of MetS (6, 8). It is therefore of crucial importance to identify key risk factors for early diagnosis and interventions.

The association of certain inflammatory cytokines and adipokines with MetS has been well documented. For instance, C-reactive protein (CRP) and adiponectin are two circulating factors that have been identified as risk predictors in association with MetS (9, 10). Retinol-binding protein 4 (RBP4), secreted by adipocytes and liver and originally known to transport retinol in circulation (11), is a newly identified adipokine that recently has been shown to contribute to insulin resistance in several mouse models (12). For instance, Yang *et al.* (12) recently found that injection of purified RBP4 into mice not only impaired insulin signaling in muscle but also increased the expression of phosphoenolpyruvate kinase in the liver, suggesting that RBP4 may serve as a therapeutic target for treatment of diabetes. Later study in human subjects indicated that elevated serum RBP4 levels were associated with the severity of insulin resistance and obesity and with certain components of MetS (13). However, many, but not all, subsequent studies confirmed the correlations of increased circulating RBP4 with various aspects of adiposity (14–20), insulin resistance (17–24), fasting glucose levels (15, 21, 25), and metabolic abnormalities such as dyslipidemia (19, 21, 24, 26). These inconsistencies most likely result from differences in age, ethnicity, sample size, and assay methods used (27). Hence, it has yet to be established whether RBP4 may serve as a risk marker for insulin resistance and type 2

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Abbreviations: BMI, Body mass index; CI, confidence interval; CRP, C-reactive protein; CVD, cardiovascular disease; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; LDL, low-density lipoprotein; MetS, metabolic syndrome; OR, odds ratio; PBST, PBS containing Tween 20; RBP4, retinol-binding protein 4.

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diabetes and to what extent it is associated with MetS and CVD.

Therefore, using a sandwich ELISA developed and validated in-house, we aimed to evaluate the RBP4 distribution in a cross-sectional population study of 3289 middle-aged and older Chinese subjects and assess whether RBP4 is independently associated with MetS and its individual components.

Subjects and Methods

Study population

The design and recruitment of the population-based cross-sectional study has been described in detail elsewhere (28). Briefly, a total of 3289 eligible participants (1458 men and 1831 women) aged 50–70 yr from urban and rural areas of north (Beijing) and south (Shanghai) China were recruited in 2005 for the Nutrition and Health of Aging Population in China study. Data on demographic variables, health status, health behavior, and physical activity were collected using a standardized questionnaire. All participants were required to fast overnight (≥7 h) before physical examination by trained staff and physicians using standard protocols. Measurements of body weight and height, waist and hip circumference, and blood pressure have been described previously (28). The study was approved by the Institutional Review Board of the Institute for Nutritional Sciences, and written informed consents were obtained from all participants.

Laboratory measurements

Overnight fasting blood samples were collected in tubes containing liquid EDTA, centrifuged at 4 C, and stored at −80 C until analysis. The measurements of total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, glucose, CRP, and adiponectin were previously described (28, 29). Insulin was measured with completely homologous radioimmunology assay (Linco Research, St. Charles, MO), which has less than 0.2% cross-reactivity with proinsulin.

RBP4 was measured in duplicate by a sandwich ELISA developed in-house, using affinity-chromatography purified polyclonal and monoclonal antibodies generated against recombinant human RBP4 protein (for details, see supplemental data published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org). The assay system was subsequently cross-validated by Western blot analysis. The intraassay coefficient of variation was 1.8–7.6% and interassay was 3.7–8.8%. Briefly, monoclonal anti-RBP4 antibody was used to coat a 96-well microplate overnight at 4 C. Blocking was performed with 250 μl/well PBS containing 1% BSA at 37 C for 1 h. After being washed three times with PBS containing 0.05% Tween 20 (PBST), 100-μl plasma samples at a dilution of 1:1000 with PBST containing 0.1% BSA, or purified His-tagged full-length RBP4 (0.001–0.5 μg/ml) as standard were applied and incubated at 37 C for 1 h. The plates were washed three times with PBST and then incubated at 37 C for 1 h with 100 μl polyclonal anti-RBP4 antibody. After being washed three times with PBST, the plates were incubated at 37 C for 1 h with 100 μl/well detection antibody (horseradish peroxidase-conjugated antirabbit IgG; Bio-Rad Laboratories, Hercules, CA). The plates were washed three times with PBST, followed by incubation at 25 C for 20 min with 100 μl of freshly mixed (1:1) stabilized peroxide solution and chromogen solution (R&D Systems, Minneapolis, MN). For detection, ODs were measured at 450 nm after the reaction was stopped by the addition of 100 μl 1 M H_2SO_4 per well.

Definition of MetS

The MetS was defined based on the updated National Cholesterol Education Program Adult Treatment Panel III criteria for Asian-Americans (1) as presenting at least three of the following components: 1) waist circumferences 90 cm or greater in men or 80 cm or greater in women; 2) triglycerides 1.7 mmol/liter or greater; 3) HDL cholesterol less than 1.03 mmol/liter in men or less than 1.30 mmol/liter in women; 4) blood pressure 130/85 mm Hg or greater or current use of antihypertensive medications; or 5) fasting plasma glucose 5.6 mmol/liter or greater or previously diagnosed type 2 diabetes or on oral antidiabetic agents or insulin.

Statistical analysis

Normally distributed data were expressed as means ± SD, whereas variables with a skewed distribution were reported as median (interquartile range) and log transformed to approximate normality before analysis. Categorical variables were represented by frequency and percentage. Analysis of covariance for continuous variables and multivariate logistic regression analysis for categorical variables were applied for the comparison according to RBP4 quartiles. Analysis of covariance was used to compare RBP4 levels between genders and geographic locations. Correlation coefficients between RBP4 and metabolic features were calculated by partial correlation analysis on ranks (Spearman correlation). Plasma RBP4 levels were depicted according to the number of MetS components using linear regression model. Multivariate logistic regression models were used to estimate the odds ratios (ORs) for MetS and its components. Potential confounding variables including age, gender, smoking, alcohol drinking, physical activity, educational level, self-reported CVD, family history of diabetes and CVD, CRP, adiponectin, homeostatic model assessment of insulin resistance (HOMA-IR), and body mass index (BMI) were controlled in the regression models. Data management and statistical analysis were performed with Stata (version 9.2; College Station, TX). $P < 0.05$ was considered statistically significant.

Results

Characteristics of participants according to RBP4 quartiles

When analyzed by quartiles of RBP4 levels, as summarized in Table 1, the subjects with higher RBP4 were more likely to be male, Beijing and urban residents, smokers, and alcohol drinkers, with a higher education level and a family history of CVD (all $P < 0.05$). With respect to metabolic parameters, the subjects in the higher RBP4 quartiles exhibited higher levels of systolic/diastolic blood pressure, BMI, waist circumference or waist-to-hip ratio, fasting insulin, HOMA-IR, triglycerides, total cholesterol, and LDL cholesterol (all $P < 0.001$). In contrast, the subjects with higher RBP4 levels displayed lower plasma adiponectin and HDL cholesterol levels (both $P < 0.001$). However, elevated RBP4 levels showed no association with the levels of fasting glucose and CRP.

Distribution of RBP4 levels

The crude mean (SD) of plasma RBP4 concentration was 40.10 (11.75) μg/ml and 41.97 (12.23) μg/ml for men and 38.61 (11.14) μg/ml for women ($P < 0.001$), respectively. Male and Beijing participants had significantly higher RBP4 levels than female and Shanghai counterparts after adjustment for potential confounders including BMI and MetS components without central obesity (supplemental table S1).

Association between RBP4 and MetS

Partial Spearman correlation analysis demonstrated the strongest correlation between RBP4 and triglycerides among various metabolic features (Table 2).

Remarkably, plasma RBP4 levels increased gradually with increasing numbers of MetS components (Fig. 1). The mean values (SE) of RBP4 concentrations for those with none to five components were 38.28 (0.75), 39.86 (0.60), 41.05 (0.57), 43.16 (0.57), 44.90 (0.65), and 47.20 (0.90) μg/ml, respectively, after adjustment for age, gender, geographic location, lifestyle

TABLE 1. Characteristics of study participants according to RBP4 quartiles[a]

Characteristics	Q1 (n = 821) <31.98	Q2 (n = 823) 31.99–39.06	Q3 (n = 822) 39.07–46.83	Q4 (n = 823) ≥46.84	*P* value
RBP4 (μg/ml)	26.62 ± 4.60	35.38 ± 2.06	42.63 ± 2.24	55.75 ± 8.13	<0.001
Age (yr)[b]	58.6 ± 6.1	58.7 ± 6.1	58.7 ± 5.9	58.4 ± 5.9	0.783
Male[b]	294 (35.8)	332 (40.3)	388 (47.2)	444 (54.0)	<0.001
Urban residents[b]	349 (42.5)	409 (49.7)	433 (52.7)	449 (54.6)	<0.001
Residents of Beijing[b]	308 (37.5)	380 (46.2)	448 (54.5)	505 (61.4)	<0.001
Smoking (yes)	241 (29.4)	288 (35.0)	319 (38.8)	400 (48.6)	0.011
Alcohol drinking (yes)	145 (17.7)	197 (23.9)	255 (31.0)	343 (41.7)	<0.001
Education (yr)					<0.001
0–6	423 (51.5)	350 (42.5)	332 (40.4)	255 (31.0)	
7–9	247 (30.1)	279 (33.9)	284 (34.5)	362 (44.0)	
≥10	151 (18.4)	194 (23.6)	206 (25.1)	206 (25.0)	
Physical activity					0.306
Low	58 (7.1)	56 (6.8)	62 (7.5)	69 (8.4)	
Moderate	336 (40.9)	338 (41.1)	349 (42.5)	358 (43.5)	
High	427 (52.0)	429 (52.1)	411 (50.0)	396 (48.1)	
Self-reported CVD[c]	65 (7.9)	72 (8.8)	95 (11.6)	101 (12.3)	0.234
Family history of diabetes[d]	85 (10.4)	109 (13.2)	133 (16.2)	127 (15.4)	0.082
Family history of CVD[d]	149 (18.2)	189 (23.0)	182 (22.1)	221 (26.9)	0.049
SBP (mm Hg)	137.00 ± 22.13	138.43 ± 21.65	141.32 ± 22.36	143.74 ± 23.15	<0.001
DBP (mm Hg)	78.04 ± 10.34	79.09 ± 10.66	81.02 ± 10.92	82.52 ± 10.76	<0.001
BMI (kg/m^2)	23.70 ± 3.57	24.16 ± 3.59	24.89 ± 3.51	25.10 ± 3.51	<0.001
Waist circumference (cm)	80.57 ± 10.53	82.29 ± 10.16	85.31 ± 10.20	86.80 ± 10.17	<0.001
Waist to hip ratio	0.87 ± 0.07	0.88 ± 0.07	0.90 ± 0.07	0.91 ± 0.07	<0.001
Glucose (mmol/liter)	5.73 ± 1.57	5.80 ± 1.76	5.81 ± 1.59	6.04 ± 1.99	0.167
Insulin (μU/ml)	13.24 (9.38–18.58)	13.00 (9.45–17.21)	13.81 (10.16–18.70)	14.62 (10.65–20.30)	<0.001
HOMA-IR	3.31 (2.25–4.67)	3.23 (2.23–4.34)	3.42 (2.45–4.82)	3.78 (2.64–5.35)	<0.001
CRP (mg/liter)	0.64 (0.29–1.42)	0.64 (0.31–1.48)	0.69 (0.36–1.61)	0.73 (0.36–1.61)	0.680
Adiponectin (μg/ml)[e]	16.92 (9.43–25.08)	13.25 (8.44–21.53)	12.86 (7.79–20.95)	12.02 (7.10–18.76)	<0.001
Triglycerides (mmol/liter)[e]	0.85 (0.61–1.25)	1.05 (0.76–1.50)	1.14 (0.83–1.75)	1.48 (0.95–2.29)	<0.001
Total cholesterol (mmol/liter)	4.43 ± 0.93	4.63 ± 0.93	4.76 ± 0.97	4.96 ± 1.01	<0.001
LDL cholesterol (mmol/liter)	3.02 ± 0.94	3.32 ± 0.94	3.34 ± 1.01	3.46 ± 1.01	<0.001
HDL cholesterol (mmol/liter)	1.32 ± 0.34	1.28 ± 0.33	1.26 ± 0.32	1.24 ± 0.34	<0.001

SBP, systolic blood pressure; DBP, diastolic blood pressure.

[a] Data are means ± SD, median (interquartile range), or number (percent); *P* value was calculated after adjustment for age, gender, region (Beijing/Shanghai), and residence (urban/rural).

[b] Not adjusted for itself.

[c] Self-reported CVD including stroke and coronary heart disease.

[d] Parents or siblings had a history of diabetes or CVD.

[e] These variables were log transformed before analysis.

factors, education status, self-reported CVD, and family history of diabetes and CVD.

As presented in Table 3, the ORs for MetS and its components (except for hyperglycemia) were higher with increasing RBP4 quartiles ($P < 0.001$ for trend). In the highest RBP4 quartile, the ORs were 2.58 [95% confidence interval (CI) 2.08–3.20] for MetS, 5.61 (4.28–7.35) for elevated triglycerides, 2.08 (1.68–2.58) for central obesity, 1.85 (1.47–2.34) for elevated blood pressure, 1.50 (1.21–1.86) for reduced HDL cholesterol, and 1.13 (0.92–1.41) for elevated

TABLE 2. Partial Spearman correlation coefficients among RBP4, anthropometric indicators, and metabolic features[a]

	RBP4	BMI	WC	SBP	DBP	Insulin	HOMA-IR	Glucose	CRP	Adiponectin	Triglycerides	Total cholesterol	LDL cholesterol
BMI	0.13[b]												
WC	0.18[b]	0.87[b]											
SBP	0.11[b]	0.27[b]	0.26[b]										
DBP	0.14[b]	0.29[b]	0.30[b]	0.70[b]									
Insulin	0.12[b]	0.39[b]	0.41[b]	0.16[b]	0.19[b]								
HOMA-IR	0.11[b]	0.39[b]	0.42[b]	0.19[b]	0.20[b]	0.92[b]							
Glucose	0.03	0.18[b]	0.21[b]	0.17[b]	0.14[b]	0.20[b]	0.47[b]						
CRP	0.01	0.32[b]	0.36[b]	0.14[b]	0.16[b]	0.22[b]	0.25[b]	0.14[b]					
Adiponectin	−0.10[b]	−0.29[b]	−0.30[b]	−0.09[b]	−0.08[b]	−0.23[b]	−0.26[b]	−0.18[b]	−0.22[b]				
Triglycerides	0.32[b]	0.36[b]	0.41[b]	0.19[b]	0.21[b]	0.32[b]	0.35[b]	0.22[b]	0.27[b]	−0.34[b]			
Total cholesterol	0.20[b]	0.07[b]	0.11[b]	0.08[b]	0.12[b]	0.05[c]	0.12[b]	0.24[b]	0.12[b]	−0.07[b]	0.34[b]		
LDL cholesterol	0.16[b]	0.13[b]	0.16[b]	0.07[b]	0.11[b]	0.07[b]	0.13[b]	0.21[b]	0.13[b]	−0.09[b]	0.28[b]	0.92[b]	
HDL cholesterol	−0.08[b]	−0.36[b]	−0.36[b]	−0.09[b]	−0.09[b]	−0.27[b]	−0.25[b]	−0.03	−0.22[b]	0.30[b]	−0.46[b]	0.34[b]	0.21[b]

WC, Waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure.

[a] All correlation coefficients were calculated after adjustment for age, gender, region, and residence.

[b] $P < 0.001$.

[c] $P < 0.05$.

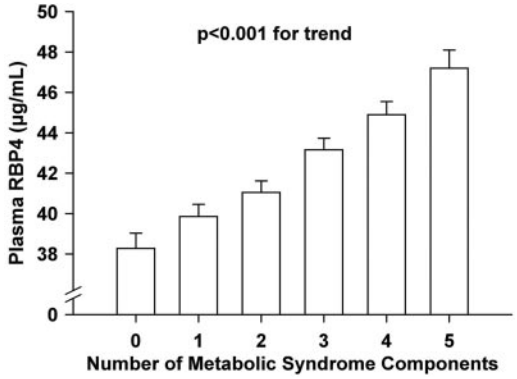

FIG. 1. Plasma RBP4 levels according to the number of MetS components. Data are shown as means ± SE after adjustment for age, gender, region, residence, alcohol drinking, smoking, educational attainment, physical activity, self-reported CVD, and family history of diabetes and CVD; $P < 0.001$ for trend.

fasting glucose after adjusting for age, gender, geographic location, lifestyle factors, educational attainment, self-reported CVD, and family history of diabetes and CVD (model 2). Interestingly, further adjustment for CRP, adiponectin, and HOMA-IR (model 3) only slightly reduced the magnitude of the ORs for MetS and its components. Furthermore, the ORs for MetS were not substantially attenuated by additional adjustment for BMI (model 4) (OR 2.25; 95% CI 1.72–2.94). In addition, when analyzed separately in men and women, the ORs for MetS were 2.79 (95% CI 1.96–3.96) in men and 2.56 (95% CI 1.93–3.40) in women with simple adjustment (model 1). In the multivariate adjusted analyses (model 2), the ORs were 2.75 (95% CI 1.92–3.94) and 2.59 (95% CI 1.94–3.45) for men and women, respectively (detail data shown in supplemental table S2).

Because increased RBP4 was associated with a particularly high risk of hypertriglyceridemia, the ORs for hypertriglyceridemia from the first to the fourth quartiles of RBP4, CRP, HOMA-IR, and adiponectin were further compared after adjustment for major confounders (Table 4). The subjects in the highest quartile had an OR of 5.61 (95% CI 4.27–7.35) with increased RBP4, 4.31 (3.29–5.64) with increased CRP, and 5.84 (4.47–7.63) with elevated HOMA-IR, whereas the subjects in the lowest adiponectin quartile had an OR of 5.54 (4.19–7.31) after controlling for age, gender, region, residence, alcohol drinking, smoking, education, physical activity, self-reported CVD, and family history of diabetes and CVD. Further adjustment for BMI (model 2) or waist circumference (model 3) reduced the risk of hypertriglyceridemia by different magnitudes. However, increased RBP4 was associated with the highest

TABLE 3. Adjusted ORs and 95% CIs for MetS and its components according to RBP4 quartiles

	ORs (95% CI)				*P* value for trend
	Q1	Q2	Q3	Q4	
MetS					
Model 1[a]	1.0	1.23 (1.00–1.52)	1.85 (1.50–2.28)	2.57 (2.08–3.18)	<0.001
Model 2[b]	1.0	1.24 (1.00–1.53)	1.84 (1.49–2.27)	2.58 (2.08–3.20)	<0.001
Model 3[c]	1.0	1.21 (0.96–1.52)	1.85 (1.47–2.33)	2.43 (1.91–3.08)	<0.001
Model 4[d]	1.0	1.15 (0.88–1.49)	1.57 (1.21–2.04)	2.25 (1.72–2.94)	<0.001
Elevated triglycerides					
Model 1[a]	1.0	1.71 (1.28–2.28)	3.00 (2.29–3.95)	5.60 (4.29–7.32)	<0.001
Model 2[b]	1.0	1.71 (1.28–2.28)	2.99 (2.27–3.93)	5.61 (4.28–7.35)	<0.001
Model 3[c]	1.0	1.64 (1.21–2.22)	2.89 (2.17–3.87)	5.32 (4.00–7.09)	<0.001
Model 4[d]	1.0	1.63 (1.20–2.21)	2.74 (2.04–3.67)	5.12 (3.83–6.84)	<0.001
Central obesity					
Model 1[a]	1.0	1.17 (0.95–1.43)	1.76 (1.43–2.16)	2.10 (1.70–2.59)	<0.001
Model 2[b]	1.0	1.17 (0.95–1.43)	1.76 (1.43–2.17)	2.08 (1.68–2.58)	<0.001
Model 3[c]	1.0	1.12 (0.90–1.39)	1.74 (1.39–2.17)	1.88 (1.50–2.37)	<0.001
Model 4[d]	1.0	0.98 (0.71–1.35)	1.34 (0.97–1.86)	1.77 (1.27–2.47)	0.001
Elevated blood pressure					
Model 1[a]	1.0	1.07 (0.87–1.33)	1.55 (1.25–1.94)	1.84 (1.47–2.32)	<0.001
Model 2[b]	1.0	1.08 (0.87–1.34)	1.55 (1.24–1.94)	1.85 (1.47–2.34)	<0.001
Model 3[c]	1.0	1.11 (0.89–1.37)	1.58 (1.26–1.98)	1.82 (1.43–2.31)	<0.001
Model 4[d]	1.0	1.08 (0.85–1.33)	1.41 (1.11–1.78)	1.65 (1.29–2.11)	<0.001
Reduced HDL cholesterol					
Model 1[a]	1.0	1.13 (0.92–1.39)	1.19 (0.96–1.46)	1.45 (1.18–1.79)	0.006
Model 2[b]	1.0	1.14 (0.93–1.40)	1.20 (0.97–1.47)	1.50 (1.21–1.86)	0.002
Model 3[c]	1.0	1.04 (0.84–1.29)	1.07 (0.86–1.33)	1.29 (1.03–1.62)	0.118
Model 4[d]	1.0	1.02 (0.82–1.27)	0.96 (0.76–1.20)	1.16 (0.92–1.46)	0.379
Elevated fasting glucose					
Model 1[a]	1.0	0.92 (0.75–1.13)	0.96 (0.78–1.18)	1.20 (0.97–1.48)	0.056
Model 2[b]	1.0	0.89 (0.72–1.10)	0.91 (0.73–1.12)	1.13 (0.92–1.41)	0.089
Model 3[c]	1.0	0.90 (0.72–1.15)	0.87 (0.69–1.10)	0.94 (0.74–1.20)	0.644
Model 4[d]	1.0	0.90 (0.71–1.13)	0.85 (0.67–1.08)	0.93 (0.73–1.18)	0.585

[a] Model 1 adjusted for age, gender, region, and residence.
[b] Model 2 further adjusted for alcohol drinking, smoking, education, physical activity, self-reported CVD, and family history of diabetes and CVD.
[c] Model 3 further adjusted for CRP, adiponectin, and HOMA-IR.
[d] Model 4 further adjusted for BMI.

TABLE 4. Adjusted ORs and 95% CIs for hypertriglyceridemia according to quartiles of RBP4, CRP, HOMA-IR, and adiponectin

	ORs (95% CI)				*P* value for trend
	Q1	Q2	Q3	Q4	
RBP4					
Model 1[a]	1.0	1.71 (1.28–2.28)	2.99 (2.27–3.93)	5.61 (4.27–7.35)	<0.001
Model 2[b]	1.0	1.66 (1.24–2.23)	2.75 (2.07–3.64)	5.17 (3.92–6.83)	<0.001
Model 3[c]	1.0	1.66 (1.24–2.24)	2.61 (1.97–3.47)	4.79 (3.62–6.34)	<0.001
CRP					
Model 1[a]	1.0	2.23 (1.28–2.28)	3.18 (2.42–4.18)	4.31 (3.29–5.64)	<0.001
Model 2[b]	1.0	2.00 (1.51–2.65)	2.43 (1.84–3.21)	2.97 (2.24–3.93)	<0.001
Model 3[c]	1.0	1.80 (1.35–2.40)	2.13 (1.61–2.83)	2.51 (1.88–3.33)	<0.001
HOMA-IR					
Model 1[a]	1.0	1.69 (1.26–2.26)	3.12 (2.37–4.11)	5.84 (4.47–7.63)	<0.001
Model 2[b]	1.0	1.49 (1.11–2.00)	2.46 (1.86–3.26)	4.03 (3.04–5.34)	<0.001
Model 3[c]	1.0	1.41 (1.05–1.90)	2.27 (1.71–3.02)	3.41 (2.56–4.53)	<0.001
Adiponectin	Q4	Q3	Q2	Q1	
Model 1[a]	1.0	2.11 (1.58–2.83)	3.09 (2.34–4.09)	5.54 (4.19–7.31)	<0.001
Model 2[b]	1.0	1.92 (1.43–2.58)	2.43 (1.82–3.24)	4.24 (3.18–5.64)	<0.001
Model 3[c]	1.0	1.88 (1.39–2.52)	2.29 (1.72–3.07)	3.98 (2.98–5.32)	<0.001

[a] Model 1 adjusted for age, gender, region, residence, alcohol drinking, smoking, education, physical activity, self-reported CVD, and family history of diabetes and CVD.
[b] Model 2 further adjusted for BMI based on model 1.
[c] Model 3 further adjusted for waist circumference based on model 1.

risk among these four parameters after these multiple adjustments.

As shown in Fig. 2, the risk for MetS increased markedly with rising levels of RBP4, higher levels of CRP (Fig. 2A), lower adiponectin levels (Fig. 2B), and higher levels of HOMA-IR (Fig. 2C). Moreover, even in the lowest quartile of CRP or HOMA-IR, and the highest quartile of adiponectin, the risks for MetS were 1- to 2-fold higher in the highest RBP4 quartile than in the lowest quartile. No significant interactions were observed on the risks for MetS between RBP4 and CRP, RBP4 and adiponectin, and RBP4 and HOMA-IR, respectively.

Discussion

We found a strong association between RBP4 levels and the risk of MetS and its key components such as central obesity, elevated triglycerides, and high blood pressure, the important risk factors for atherosclerosis and type 2 diabetes in a large-scale population study. Moreover, this association is independent of lifestyle factors, education status, geographic location, family history of chronic diseases, and, remarkably, CRP, adiponectin, HOMA-IR, and BMI.

One of the interesting findings of the present study is that the distribution of RBP4 levels in the middle-aged and elderly population in China substantially differed by gender and geographic location. Consistent with a previous report (26), the RBP4 levels were sexually dimorphic in our study population, even after adjusting for the confounders, which might be explained by the different levels of adiposity and influences of sex hormones on adipokines (29, 30). However, the differences of RBP4 levels between residents of Beijing and Shanghai could not be fully explained by the confounding variables. Nonetheless, it is unknown whether other potential factors such as retinol or transthyretin, which forms as a complex with RBP4 in the circulation, could also modulate plasma RBP4 levels in this population (11, 31). Indeed, a recent study suggested that alterations in transthyretin may contribute to elevated RBP4 in obese individuals (32).

High CRP and low adiponectin levels are well-documented risk factors for MetS and its components (9, 10). However, the relationship between RBP4 and CRP or adiponectin has not been well addressed in a large population study. Balagopal *et al.* (18) reported that RBP4 was associated positively with CRP and negatively with adiponectin in a small group of obese children, whereas no correlation between RBP4 and CRP or adiponectin was observed by Takebayashi *et al.* (24) in a study of 101 hospitalized type 2 diabetic patients. With a larger sample size, we found a weak inverse correlation between RBP4 and adiponectin levels, but no correlation was detected between RBP4 and CRP levels. Interestingly, although high levels of CRP and RBP4 or hypoadiponectinemia were closely associated with an increased risk of MetS, increased RBP4 *per se* was an independent risk factor for the MetS, even within the lowest CRP or the highest adiponectin quartile. Therefore, it is possible that RBP4 may promote the MetS risk through a pathway not fully overlapping with CRP or adiponectin.

Consistent with recent publications (15, 26), we found that elevated RBP4 levels were associated with insulin resistance assessed by HOMA-IR. However, our study showed that RBP4 levels were not correlated with fasting glucose or type 2 diabetes, which agrees with some recent studies (15, 21, 25) but not others (26, 24). The discrepancies in different studies may be due to variations in participant characteristics, sample sizes, or measurement methods. In addition, despite the fact that insulin resistance has been considered a major contributor to MetS (6, 7), here we found that the subjects in the highest RBP4 quartile showed a MetS risk more than twice as much as that for those in the lowest quartile, regardless of the degree of HOMA-IR, suggesting that an elevated RBP4 level alone may enhance the MetS phenotype via mechanism(s) other than insulin resistance.

It is worth noting from our study that the association

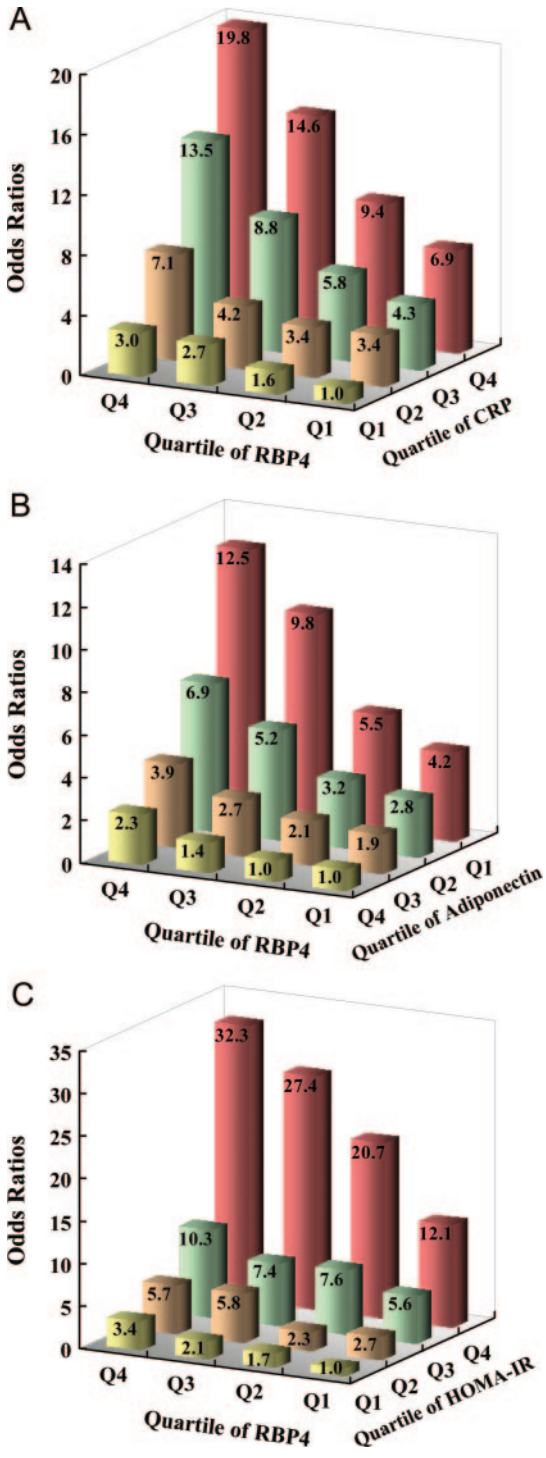

FIG. 2. Adjusted ORs for MetS according to the quartiles of RBP4 and CRP (A), RBP4 and adiponectin (B), and RBP4 and HOMA-IR (C). Adjusted for age, gender, region, residence, alcohol drinking, smoking, educational attainment, physical activity, self-reported CVD, and family history of diabetes and CVD ($P = 0.618$ for interaction of RBP4 and CRP, $P = 0.916$ for interaction of RBP4 and adiponectin, and $P = 0.092$ for interaction of RBP4 and HOMA-IR).

between RBP4 and hypertriglyceridemia was particularly strong, compared with other features of MetS. This close association was consistent with previous observations in subjects with either normal or abnormal glucose tolerance (21, 24, 25). In addition, the fact that RBP4 levels correlated with hypertriglyceridemia independently of insulin resistance is in agreement with previous studies in family members of subjects with type 2 diabetes (13). Moreover, compared with the associations of hypertriglyceridemia with CRP, adiponectin, or HOMA-IR, this association with RBP4 was less influenced by multiple adjustments for BMI or waist circumference, which are well-known risk factors for hypertriglyceridemia (33, 34). Atherogenic dyslipidemia, such as hypertriglyceridemia, has been considered an independent risk factor for CVD (35). However, it remains unclear whether elevated RBP4 expression directly or indirectly leads to dysregulated lipid metabolism or vice versa. Given the basic function of RBP4 as a retinol-binding protein, it is reasonable to speculate that RBP4 may serve as a link between retinol metabolism and activation of nuclear receptors (such as retinoic acid receptors and retinoic acid-X receptors) and may also play a role in the regulation of lipid homeostasis. Indeed, administration of 13-*cis*-retinoic acid resulted in an increased risk of dyslipidemia and MetS in humans (36, 37). On the other hand, it is also possible that hypertriglyceridemia accompanied by hyperinsulinemia may trigger RBP4 synthesis and secretion in liver or ectopic fat because triglyceride surplus may deposit in the liver as ectopic fat (38), and circulating RBP4 was reported to be closely related with liver fat (15). Together, the association between RBP4 and hypertriglyceridemia as well as MetS may provide new insights into the potential involvement of retinol metabolism in the pathogenesis of MetS and related disorders.

Obesity and visceral adiposity tend to be the most prevalent features of the MetS (38), and RBP4 levels have been reported to be highly correlated with BMI and waist circumference, which was confirmed in our study. However, adjustment for BMI yielded only a minor reduction of the MetS risk across the RBP4 quartiles. Thus, high circulating levels of RBP4 in the individuals with high MetS risk may not be merely a consequence of excess adipose tissue. Indeed, Janke *et al.* (14) reported that serum RBP4 levels were similar in normal-weight, overweight, and obese women. Furthermore, a recent study showed that elevation in circulating RBP4 in health human subjects was only associated with liver fat (15). Previous studies in rodents also revealed a higher level of RBP4 mRNA in the liver than in adipose tissues under normal conditions (39), whereas increased RBP4 expression in adipose tissues, but not in the liver, was observed in insulin-resistant mice (12). Despite the fact that visceral adipose tissue has been suggested to be a major source of RBP4 in insulin-resistant states (32), it remains unknown whether metabolic disorders in humans are associated with elevated hepatic RBP4 expression or secretion.

MetS is a well-established risk factor for type 2 diabetes and CVD in both Western (3) and Chinese populations (40). Graham *et al.* (13) demonstrated that RBP4 levels increased before full-blown diabetes manifested. However, whether higher levels of RBP4 actually predict type 2 diabetes and CVD cannot be determined through the present study. Nevertheless, given the close association among RBP4 levels and dyslipidemia, insulin resistance, and other metabolic disorders together with the findings that serum RBP4 levels were

reduced after exercise training (13), weight loss (16), and lifestyle intervention (18) in individuals with obesity or insulin resistance, it is plausible to consider RBP4 as a promising candidate for risk assessment and a potential target for intervention. Certainly, prospective studies with solid clinical end points are urgently needed to clarify whether a high RBP4 level plays a causal role in the development of MetS, type 2 diabetes, and CVD.

To our knowledge, this is the first study to evaluate the relationship between RBP4 levels and MetS in a large-scale population. Most potential confounders were carefully controlled, which limited the possibility of residual confounding effects. Furthermore, RBP4 concentrations were measured in duplicate, and the field study was completed within 2 months to minimize seasonal influences on biomarkers and other lifestyle factors. However, due to the cross-sectional nature of the present study, admittedly, we could not determine whether RBP4 plays a causal role in the pathogenesis of MetS. Also, it has yet to be seen whether our results in middle-aged and older Chinese subjects can be generalized to younger populations or other ethnic groups.

In conclusion, we have found that elevated RBP4 levels are strongly and independently associated with MetS. Although longitudinal studies are needed, our findings provide novel insights into the potential role of RBP4 in the pathogenesis of MetS, type 2 diabetes, and CVD as well as in the prevention and management of these metabolic diseases.

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