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Levels of retinol-binding protein 4 and uric acid in patients with type 2 diabetes mellitus

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Abstract

Retinol-binding protein 4 (RBP4) is a novel adipocytokine. It was observed that retinoid intoxication was related to acute attacks of gout. Furthermore, animal study has shown that colchicine inhibits RBP secretion. The aim of this study was to explore the association between RBP4 level and metabolic parameters especially uric acid in patients with type 2 diabetes mellitus. Serum RBP4 level was measured by a commercial competitive enzyme-linked immunosorbent assay kit, and its correlation with clinical and metabolic parameters was analyzed. Data on 885 subjects were used in the analysis. Pearson correlations revealed that serum RBP4 level correlated positively with age, waist circumference, waist-to-hip ratio, systolic blood pressure, total cholesterol, triglyceride, uric acid, creatinine, and urine albumin-to-creatinine ratio. Serum RBP4 level correlated negatively with estimated glomerular filtration rate but did not correlate with body mass index, homeostasis model assessment, A_{1C}, or high-sensitivity C-reactive protein. Multiple linear regression analysis with serum RBP4 level as the dependent variable revealed that total cholesterol, triglyceride, uric acid, and albumin-to-creatinine ratio correlated independently and positively with serum RBP4 level and that estimated glomerular filtration rate correlated independently and negatively with serum RBP4 level. In conclusion, RBP4 level was independently associated with uric acid level.
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1. Introduction

Vitamin A is a generic term for a group of compounds with similar biologic activity, such as retinol, retinal, and retinoic acid. The term *retinoids* is a collective term for these 3 compounds and other synthetic compounds with vitamin A–like activity. Retinol-binding protein (RBP), a transport protein for vitamin A (retinol), is a single polypeptide chain with a molecular weight of about 21 kd, synthesized mainly by the hepatocyte and secreted into the circulation bound to retinol and transthyretin (formerly called *prealbumin*) to form a greater–molecular weight (about 85 kd) retinol-RBP-transthyretin complex to deliver retinol to tissues [1]. In 2005, Yang et al [2] reported that RBP4 is a novel adipocyte-secreted hormone that is up-regulated in several insulin-resistant mouse models. Studies in humans have shown that RBP4 level is higher in patients with obesity, type 2 diabetes mellitus (DM), metabolic syndrome, and polycystic ovary syndrome than in control subjects [2-6]. Serum RBP4 level has also been shown to be positively associated with insulin resistance [3]. Peroxisome proliferator–activated receptor γ agonist, an insulin sensitizer, reduces serum RBP4 level in patients with type 2 DM [7]. Although some studies have shown that RBP4 is a useful biomarker of insulin resistance among individuals with a variety of clinical presentations, others have not found evidence to support such a correlation [8-10]. Thus, the relationship between RBP4 level and insulin resistance is controversial.

Previous reports have shown that uric acid is one of the components of insulin resistance [11,12] and could serve as a

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simple biomarker of insulin resistance [11]. Furthermore, it has been observed that retinol intoxication can trigger acute gout attacks [13,14]. Colchicine has been shown to inhibit RBP secretion in an animal model [15]. Few studies have investigated the relationship between uric acid level and RBP4 level. This study aimed to clarify the association between RBP4 level and metabolic parameters, especially uric acid levels, in patients with type 2 DM.

2. Materials and methods

2.1. Subjects

The study populations comprised patients who participated in our Type 2 DM Genetic Study on Non-aboriginal Taiwanese. The diagnosis of type 2 DM was based on the criteria of the American Diabetes Association [16]. Patients with age at onset less than 35 years old, history of cancer, liver cirrhosis, hyperthyroidism, and steroid use were excluded from the current study. Informed consent was obtained from each participant, and the study was approved by the Human Research Committee of the China Medical University Hospital. Blood samples were drawn between 8:00 and 10:00 AM subsequent to an overnight fast, and the separated serum was stored at −70°C until assayed for RBP4 and insulin levels. Data on 885 patients were used in the analysis.

2.2. Laboratory analysis

Plasma glucose level was determined by a hexokinase method (Integra 700; Roche, Mannheim, Germany), and the

Table 1
Clinical and biochemical characteristics of the study patients

Variables	All (N = 885)	Men (n = 440)	Women (n = 445)	*P* value
	Mean ± SD	Mean ± SD	Mean ± SD	
Age (y)	60.85 ± 10.54	60.49 ± 10.90	61.19 ± 10.18	.324
Sex (M/F)	440/445	–	–	–
DM duration (y)	9.48 ± 7.19	9.46 ± 7.29	9.50 ± 7.10	.942
Adiposity index				
Body mass index (kg/m^2)	25.62 ± 3.79	25.52 ± 3.47	25.72 ± 4.07	.436
Waist circumference (cm)	89.09 ± 9.95	90.30 ± 8.81	87.89 ± 10.83	<.001
Waist-hip ratio	0.92 ± 0.06	0.93 ± 0.05	0.91 ± 0.07	<.001
Blood pressure (mm Hg)				
Systolic	130 ± 18	129 ± 16	131 ± 20	.324
Diastolic	76 ± 11	78 ± 11	73 ± 11	<.001
Glucose-related index				
FPG (mmol/L)	7.94 ± 2.17	8.01 ± 2.19	7.86 ± 2.15	.310
FSI (pmol/L)[b]	111.58 ± 83.74	103.79 ± 74.95	119.28 ± 91.04	.006
HOMA[b]	5.56 ± 4.96	5.21 ± 4.57	5.91 ± 5.30	.036
A_{1C} (%)	7.88 ± 1.42	7.83 ± 1.44	7.94 ± 1.40	.262
Lipid profile				
Total cholesterol (mmol/L)	4.85 ± 1.03	4.73 ± 0.98	4.98 ± 1.06	<.001
Triglyceride (mmol/L)	18.45 ± 13.44	19.28 ± 15.82	17.63 ± 10.53	.069
HDL-C (mmol/L)	1.26 ± 0.35	1.17 ± 0.33	1.34 ± 0.36	<.001
LDL-C (mmol/L)	3.08 ± 0.95	3.00 ± 0.92	3.15 ± 0.98	.027
Renal index				
ACR (mg/g creatinine)[a]	17.70 (7.8-63.56)	16.67 (7.00-59.10)	19.10 (9.77-68.30)	.050
Creatinine (μmol/L)	79.99 ± 58.01	91.11 ± 63.36	69.00 ± 49.88	<.001
eGFR (mL/[min 1.73m^2])	94.79 ± 31.97	90.06 ± 27.09	99.46 ± 35.58	<.001
Uric acid (μmol/L)	372.30 ± 105.35	387.01 ± 102.61	357.76 ± 106.13	<.001
hs-CRP (mg/dL)	0.26 ± 0.42	0.25 ± 0.46	0.27 ± 0.38	.643
RBP4 (μg/mL)	17.90 ± 6.37	18.15 ± 6.18	17.65 ± 6.55	.240
Medications				
Insulin secretagogues	655 (74.01%)	333 (75.68%)	322 (72.36%)	.260
Biguanide	541 (61.13%)	273 (62.05%)	268 (60.22%)	.579
α-Glucosidase inhibitor	126 (14.24%)	60 (13.64%)	66 (14.83%)	.611
Thiazolidinedione	144 (16.27%)	75 (17.05%)	69 (15.51%)	.535
Insulin	138 (15.59%)	58 (13.18%)	80 (17.98%)	.049
ACEI or ARB	320 (36.16%)	171 (38.86%)	149 (33.48%)	.096
Hypouricemic agent	31 (3.50%)	26 (5.91%)	5 (1.12%)	<.001
Diuretic	225 (25.42%)	107 (24.32%)	118 (26.52%)	.453

Data are mean ± SD or median (interquartile range) for continuous variables or number (percentage) for categorical variables. FPG indicates fasting plasma glucose; FSI, fasting serum insulin; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blockage.

[a] Albumin-to-creatinine ratio = median (interquartile range).

[b] Fasting serum insulin and HOMA excluded insulin-treated patients.

Table 2
Pearson correlations between RBP4 level and variables

Variables	*r*	*P* value
Age (y)	0.12	<.001
Body mass index	0.02	–
Waist circumference	0.09	.011
Waist-hip ratio	0.13	<.001
Systolic blood pressure	0.16	<.001
Diastolic blood pressure	0.03	–
Fasting plasma glucose	−0.07	–
Fasting serum insulin	0.02	–
HOMA	−0.01	–
A_{1C}	−0.01	–
Total cholesterol	0.17	<.001
Triglyceride	0.21	<.001
HDL-C	−0.01	–
LDL-C	0.05	–
Uric acid	0.34	<.001
Creatinine	0.43	<.001
ACR	0.30	<.001
eGFR	−0.40	<.001
hs-CRP	−0.02	–

serum insulin level was measured by a commercial radioimmunoassay method (Adaltis Italia, Reno, Italy). The insulin resistance index from fasting serum insulin and plasma glucose levels was estimated by homeostasis model assessment (HOMA) [17]. The A_{1C} level was measured by a high-performance liquid chromatography method (HLC-723G7; TOSOH Bioscience, Tokyo, Japan). Urine albumin and high-sensitivity C-reactive protein (hs-CRP) were measured by an immunoturbidimetry method (Integra 700, Roche). Morning spot urine was used to measure albumin-to-creatinine ratio (ACR). The estimated glomerular filtration rate (eGFR) was calculated by the Modification of Diet in Renal Disease equation, where eGFR = 186.3 × [serum creatinine (in milligrams per deciliter)]$^{-1.154}$ × (age)$^{-0.203}$ × (0.742 if female) [18]. Serum cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), creatinine, and uric acid levels were determined by standard laboratory methods (Integra 700, Roche). Serum RBP4 level was measured by a commercial competitive enzyme-linked immunoassay (Phoenix Pharmaceuticals, Belmont, CA). The limit of detection was 2.40 μg/mL; and the intraassay and interassay coefficients of variation were 4.78% and 10.52%, respectively.

2.3. Statistical analysis

Data are presented as mean ± standard deviation (SD) or median (interquartile range) for continuous variables or number (percentage) for categorical variables. The differences of continuous variables presented as mean ± SD between sexes were compared by Student *t* test, and those presented as median (interquartile range) were analyzed by Wilcoxon 2-sample test. Medication differences between sexes were determined by χ^2 test. The level of correlation between serum RBP4 level and various parameters was assessed by Pearson correlations. Multiple linear regression analysis was used to identify the independent contributions of each parameter on serum RBP4 level. A *P* value of less than .05 represented statistical significance in all analyses. All statistical analyses were performed on a personal computer with the statistical package SAS for Windows (Version 9.1; SAS Institute, Cary, NC).

3. Results

Table 1 shows the clinical characteristics of the study patients. Mean waist circumference, waist-to-hip ratio, and diastolic blood pressure values were significantly higher in men than in women. Fasting serum insulin level and HOMA index were significantly higher in women than in men. Women also had significantly higher levels of total cholesterol, HDL-C, LDL-C, and eGFR and significantly lower levels of creatinine and uric acid than men. Use of hypouricemic agents was significantly more common among men. More women were treated with insulin than men.

Pearson correlations (Table 2) revealed that serum RBP4 level correlated positively with age, waist circumference, waist-to-hip ratio, systolic blood pressure, total cholesterol, triglyceride, uric acid (r = 0.34, P < .001), creatinine (r = 0.43, P < .001), and urine ACR (r = 0.30, P < .001). Serum RBP4 level correlated negatively with eGFR (r = −0.40, P < .001) but did not correlate with BMI (P = .525), HOMA (P = .833), A_{1C} (P = .867), or hs-CRP (P = .794). Multiple linear regression analysis with serum RBP4 level as the dependent variable revealed that total cholesterol (β = .742, P < .001), triglyceride (β = .042, P < .001), uric acid (β = .010, P <

Table 3
Multiple linear regression analysis using RBP4 level as the dependent variable

Variable	EC (β)	SE	*t* value	*P* value
Intercept	13.735	2.972	4.620	<.001
Age	0.011	0.020	0.540	.592
Waist circumference	−0.018	0.020	−0.930	.351
Systolic blood pressure	0.006	0.011	0.540	.590
Total cholesterol	0.742	0.204	3.640	<.001
Triglyceride	0.042	0.016	2.620	<.001
Uric acid	0.010	0.002	4.610	<.001
ACR	0.002	0.000	6.230	<.001
eGFR	−0.045	0.008	−5.780	<.001
Sex	0.038	0.385	0.100	.921
Thiazolidinedione	0.320	0.493	0.650	.516
ACEI	0.997	0.524	1.900	.057
ARB	0.411	0.527	0.780	.436
Hypouricemic agent	−0.333	1.057	−0.310	.753
Diuretic	−0.341	0.483	−0.700	.481

Body mass index and waist-to-hip ratio were not included in the model because of high degree of colinearity with waist circumference. The same reason exists between creatinine and eGFR. EC indicates estimate coefficient; SE, standard error.

.001), and ACR ($\beta = .002$, $P < .001$) correlated independently and positively with serum RBP4 level and that eGFR ($\beta = -0.045$, $P < .001$) correlated independently and negatively with serum RBP4 level (Table 3).

4. Discussion

The results of this study clearly show that serum RBP4 level is influenced by renal function and albumuria. Among the metabolic parameters, RBP4 level was independently associated with uric acid level after controlling for urine albumin level and estimated renal function. The RBP4 level was also related to total cholesterol and triglyceride levels but not related to HOMA, a finding consistent with that reported in other studies [8-10] but inconsistent with the original report of the contribution of RBP4 to insulin resistance [2].

Retinol-binding protein 4 level has been shown to be inversely correlated with eGFR level in patients with type 2 DM [19]. In addition, increased RBP4 level has been reported in patients on long-term hemodialysis [20] and in type 2 DM patients with microalbuminuria [21]. Henze et al [22] demonstrated that RBP4 level is related to renal function rather than type 2 DM. The results of our study are consistent with those reports. Because uric acid is excreted by the kidney, we can expect an inverse association between uric acid level and eGFR. Logically, the correlation between uric acid level and RBP4 level depends on eGFR; however, after adjusting for microalbuminuria and eGFR, uric acid was still independently related to RBP4 level. This result is consistent with a previous report [23]. The mechanism to explain the correlation between RBP4 level and uric acid level is still unclear at this moment. There is some evidence that supports a linkage between uric acid and retinoids. For example, one study reported that some patients developed hyperuricemia after receiving oral doses of the synthetic retinoid isotretinoin (40-80 mg/d) for treatment of severe acne [24]. Furthermore, in an animal model, colchicine treatment markedly inhibited the retinol-stimulated secretion of RBP from liver to serum [15]. These findings imply that *retinoids or RBP* may be involved in uric acid metabolism. Manipulation of RBP4 level may be a therapeutic strategy for treating hyperuricemia. Fenretinide, another synthetic derivative of vitamin A, was found to reduce RBP4 levels [25]. This derivative may be a potential insulin sensitizer and hypouricemic agent. However, because no studies have demonstrated a causal relationship between RBP4 and uric acid so far and because dietary vitamin A intoxication differs from intoxication with synthetic retinoids, further studies are necessary to support this speculation.

In conclusion, our results show that RBP4 level correlates with uric acid level. Further studies are needed to investigate the linkage between RBP4 and uric acid and to determine whether hypouricemic agents can modulate RBP4 level.

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