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Elevated serum retinol-binding protein 4 is associated with insulin resistance in older women

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Abstract

Retinol-binding protein 4 (RBP4), a molecule secreted from adipocytes and hepatocytes, may contribute to insulin resistance and is a potential predictor for type 2 diabetes mellitus. We investigated the association between serum RBP4 concentrations and insulin resistance in perimenopausal women. In addition, we examined associations of serum RBP4 concentrations with age, risk factors of cardiovascular disease, and metabolic syndrome. A total of 73 healthy women were included in this study. Subjects' anthropometric measurements were taken, and body mass index and waist-hip ratio were calculated. Fasting glucose, fasting insulin, serum RBP4, and lipid parameters were examined. These various parameters were compared in subjects younger than and older than 50 years. Serum RBP4 concentrations in women at least 50 years of age were significantly higher than those in women younger than 50 years. In all subjects, serum RBP4 concentrations positively correlated with age, diastolic blood pressure, fasting glucose, and homeostatic assessment model of insulin resistance. After subgroup analysis, serum RBP4 concentrations positively correlated with age, fasting glucose, and homeostatic assessment model of insulin resistance in women at least 50 years of age. In women younger than 50 years, serum RBP4 concentrations positively correlated only with fasting glucose. Serum RBP4 appears to identify age-induced insulin resistance by physiologic changes due to aging or menopause and by increasing hepatic glucose production. However, the clinical implication of RBP4 for detecting cardiovascular disease and metabolic syndrome is not clear.

1. Introduction

Insulin resistance (IR) has a causal role in the development of type 2 diabetes mellitus [1] and is an early and strong determinant of type 2 diabetes mellitus [2-4]. Even in the absence of hyperglycemia or diabetes, IR contributes to an increased risk of cardiovascular disease [5] and constitutes a pathophysiologic link between obesity and atherosclerosis [6-8]. Early detection of IR is important to provide proper management and to predict the development of type 2 diabetes mellitus and/or cardiovascular disease.

Obesity is a major cause of IR [9], but IR does not develop in all obese people. Genetic background may play a role in IR, even in those who are nonobese [10]. The prevalence of type 2 diabetes mellitus and impaired glucose tolerance (IGT) increases with age [11]. Age is a strong risk factor for the development of IR and diabetes [12].

Adipose tissue secretes various adipokines involved in IR [13]. Studies in mice suggest that adipose tissue serves as a glucose sensor and regulates systemic glucose metabolism through the release of a circulating factor in response to decreased intracellular glucose concentrations [14]. A subsequent study revealed that the adipose-derived circulating factor is retinol-binding protein 4 (RBP4) [15]. Retinol-binding protein 4 modulates glucose metabolism and, consequently, induces IR [16]. Serum RBP4 concentrations correlate with the magnitude of IR in people who are obese and have IGT or type 2 diabetes mellitus, and

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also in nonobese, nondiabetic people with a strong family history of type 2 diabetes mellitus [17]. Elevated serum RBP4 concentrations have a significant association with cardiovascular disease risk factors and metabolic syndrome [18].

It has been proposed that RBP4 induces IR and glucose intolerance. In skeletal muscle, RBP4 reduces insulin sensitivity by inhibiting both insulin receptor substrate–1 phosphorylation and phosphatidylinositol 3-kinase activation, while increasing the rate of hepatic glucose production by increasing the activity of the gluconeogenic enzyme phosphoenolpyruvate carboxykinase [15].

However, the clinical implications of RBP4 in humans are unclear. Studies on humans have tended to involve a heterogenous group composed of obese individuals and individuals with glucose intolerance or type 2 diabetes mellitus. Here, we evaluated the relationship of RBP4 to IR in perimenopausal women who were at an increased risk for cardiovascular disease and metabolic syndrome. In addition, we investigated the association between RBP4 and cardiovascular disease risk factors, metabolic syndrome, and age.

2. Subjects and methods

2.1. Subjects

Ninety-two women who underwent a physical and medical examination at the Guro Public Medical Center were recruited. General health parameters assessed included screening history, physical examination, blood tests, and imaging tests. Individuals with a family history or a diagnosis of diabetes, or those who took any medications that would affect IR or metabolism were excluded. The final total number of participants was 73. None of the women were smokers. A questionnaire was used to assess past and/or current medical illness of the study subjects. All subjects signed an informed consent approved by the hospital's ethical committee.

2.2. Methods

Each (lightly clothed) subject underwent anthropometric evaluation, with height and weight measured by an automatic height-weight scale, from which a body mass index (BMI) was calculated. Waist circumference was measured at the midpoint between the lower border of the rib edge and the iliac crest. Hip circumference was measured at the widest part of the hip region. Blood pressure was measured by a mercury sphygmomanometer after the patient had been sitting in a chair for 10 minutes. To reduce variation, 1 person recorded all of the anthropometric measurements. Blood samples were obtained after an overnight fasting. Blood measurements included fasting glucose, fasting insulin, total cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglyceride concentrations. Low-density lipoprotein cholesterol (LDL-C) was calculated by the Friedewald equation, as follows: LDL-C = total cholesterol − [HDLC + triglycerides/5].

Insulin resistance was estimated using the homeostatic model assessment of IR (HOMA-IR), as follows: HOMA-IR = [fasting insulin (in micro–international units per milliliter)× fasting glucose (in milligrams per deciliter)/18]/22.5. Serum RBP4 levels were measured using a human RBP4 enzyme-linked immunosorbent assay kit (Adipogen, Seoul, South Korea) with an intraassay coefficient of variation of 1·5%. To evaluate the differences in serum RBP4 concentrations according to age, subjects were divided into 2 groups based on age(<50 and ≥50 years). The use of 50 years was an arbitrary choice in the absence of data concerning menopausal status and reflected the observation that the average menopausal age of Korean women is 49.2 years [19]. The association between serum RBP4 concentrations and other parameters in all subjects was assessed in the 2 age groups.

2.3. Statistical analyses

Data are presented as mean ± SD. Variables such as RBP4, triglycerides, HDL-C, and HOMA-IR concentrations were logarithmically transformed before statistical analysis to approximate normal distribution. However, the mean values of the variables are presented with untransformed data. Differences between groups were analyzed with Student t test. Pearson correlations were used to evaluate the relationship between RBP4 concentrations and clinical and metabolic variables. Simple linear regression and multiple linear regression analyses were performed to determine the association between RBP4 concentrations and the explanatory variables of age, BMI, waist circumference, systolic blood pressure, diastolic blood pressure, fasting insulin, HOMA-IR, total cholesterol, HDL-C, and triglyceride concentrations. All analyses were performed using SPSS software, version 12.0, (SPSS, Chicago, IL). Results were considered significant when $P < .05$.

3. Results

3.1. Serum RBP4 concentrations and other clinical characteristics according to age

The serum RBP4 concentrations and other clinical characteristics are shown in Table 1. Serum RBP4 concentrations were significantly higher in women at least 50 years of age than in women younger than 50 years. Women at least 50 years of age also displayed significantly higher systolic and diastolic blood pressures. There was no significant difference in the mean level of other clinical characteristics between the 2 age groups.

Table 1
Clinical and metabolic variables according to age

	Age <50 y (n = 43)	Age ≥50 y (n = 30)	P
Age (y)	44.7 ± 3.6	53.5 ± 1.6	<.001
BMI (kg/m^2)	26.8 ± 2.8	25.9 ± 2.5	.18
Waist (cm)	84.0 ± 6.1	85.4 ± 5.4	.32
WHR	0.86 ± 0.05	0.88 ± 0.04	.09
Systolic (mm Hg)	120.6 ± 16.1	131.0 ± 13.7	.006
Diastolic (mm Hg)	78.3 ± 9.9	85.0 ± 12.9	.014
Fasting glucose (mg/dL)	81.9 ± 9.6	85.6 ± 12.6	.17
Fasting insulin (μIU/mL)	12.2 ± 6.6	12.4 ± 5.8	.89
HOMA-IR	2.50 ± 1.39	2.61 ± 1.31	.54
RBP4 (μg/mL)	21.8 ± 14.8	35.3 ± 31.5	.04
Total cholesterol (mg/dL)	194.0 ± 33.2	205.8 ± 35.9	.16
Triglyceride (mg/dL)	107.6 ± 60.4	100.7 ± 59.6	.55
HDL (mg/dL)	54.6 ± 21.6	62.6 ± 27.8	.20
LDL (mg/dL)	117.9 ± 37.0	123.0 ± 40.1	.58
Fat (%)	36.4 ± 4.9	36.4 ± 4.7	.99

Data are mean ± SD. The RBP4, triglycerides, HDL-C, and HOMA-IR concentrations were logarithmically transformed before statistical analysis to approximate normal distribution. However, untransformed data are shown in the table. *P* values were calculated by Student *t* test.

3.2. Correlation between serum RBP4 concentrations and IR and other clinical characteristics

Serum RBP4 concentrations were positively correlated with age, diastolic blood pressure, fasting glucose, and HOMA-IR (Table 2). After subgroup analysis, serum RBP4 concentrations positively correlated with age, fasting glucose, and HOMA-IR in women at least 50 years of age (Table 3). In women younger than 50 years, serum RBP4 levels positively correlated with only fasting glucose (Table 3).

Table 2
Correlation between RBP4 concentrations and various parameters

	RBP4	
	r	*P*
Age (y)	0.32	.007
BMI (kg/m^2)	−0.04	.73
Waist (cm)	−0.07	.57
WHR	−0.06	.59
Systolic (mm Hg)	0.13	.28
Diastolic (mm Hg)	0.28	.017
Fasting glucose (mg/dL)	0.52	<.001
Fasting insulin (μIU/mL)	0.14	.23
HOMA-IR	0.31	.009
Total cholesterol (mg/dL)	0.04	.73
Triglyceride (mg/dL)	0.17	.15
HDL (mg/dL)	0.01	.92
LDL (mg/dL)	−0.04	.97
Fat (%)	−0.17	.19

Coefficients (*r*) and *P* values were calculated by the Pearson correlation analysis. The RBP4, triglycerides, HDL-C, and HOMA-IR concentrations were logarithmically transformed before statistical analysis to approximate normal distribution.

Table 3
Correlation between RBP4 concentrations and various parameters after subgroup analysis

	Age <50 y		Age ≥50 y	
	RBP4		RBP4	
	r	*P*	*R*	*P*
Age (y)	0.16	.32	0.37	.046
BMI (kg/m^2)	−0.01	.99	−0.01	.99
Waist (cm)	−0.08	.63	−0.14	.47
WHR	−0.35	.82	−0.24	.21
Systolic (mm Hg)	0.12	.44	−0.03	.90
Diastolic (mm Hg)	0.18	.24	0.25	.18
Fasting glucose (mg/dL)	0.54	<.001	0.46	<.001
Fasting insulin (μIU/mL)	0.04	.81	0.27	.15
HOMA-IR	0.23	.13	0.40	.029
Total cholesterol (mg/dL)	0.20	.20	−0.18	.38
Triglyceride (mg/dL)	0.25	.11	0.15	.43
HDL (mg/dL)	0.000	.998	−0.05	.78
LDL (mg/dL)	0.14	.38	−0.17	.38
Fat(%)	−0.29	.08	−0.09	.66

Coefficients (*r*) and *P* values were calculated by the Pearson correlation analysis. The RBP4, triglycerides, HDL-C, and HOMA-IR concentrations were logarithmically transformed before statistical analysis to approximate normal distribution.

3.3. Simple linear regression analysis for the relationship between RBP4 and clinical variables

Simple linear regression revealed that fasting glucose was independently associated with serum RBP4 concentrations in all subjects. Fasting glucose was also associated with serum RBP4 concentrations in both age groups (Table 4).

3.4. Multiple linear regression analysis for the relationship between RBP4 and clinical variables

Multiple linear regression analysis revealed that age, fasting insulin, and HOMA-IR were independently associated with serum RBP4 concentrations in all subjects. In addition, HOMA-IR was independently associated with

Table 4
Simple linear regression analysis to assess relationship between RBP4 and clinical variables

	Unstandardized coefficients		Standardized coefficients	
	β	SE	β	*P*
All subjects				
Fasting glucose	0.010	0.003	0.402	.001
Age <50 y				
Fasting glucose	0.012	0.003	0.543	.000
Age ≥50 y				
Fasting glucose	0.009	0.004	0.361	.043

In this simple linear analysis, variables significantly associated with RBP4 (<.05) are shown. The RBP4, triglycerides, HDL-C, and HOMA-IR concentrations were logarithmically transformed before statistical analysis to approximate normal distribution.

Table 5
Multiple regression analysis to assess relationship between RBP4 and clinical variables

	Unstandardized coefficients		Standardized coefficients	
	β	SE	β	P
All subjects				
Age	0.012	0.006	0.232	.036
Fasting insulin	−0.027	0.010	−0.610	.014
HOMA-IR	0.881	0.266	0.807	.001
Age ≥50 y				
HOMA-IR	0.578	0.251	0.399	.029

In this multiple analysis, variables significantly associated with RBP4 (<.05) are shown. The RBP4, triglycerides, HDL-C, and HOMA-IR concentrations were logarithmically transformed before statistical analysis to approximate normal distribution.

serum RBP4 concentrations in women at least 50 years of age (Table 5).

4. Discussion

Retinol-binding protein 4 is an adipokine secreted by adipocytes and hepatocytes that was recently implicated as a link between obesity and IR [16]. This discovery suggested the possibilities for antidiabetic therapies aimed at lowering serum RBP4 concentrations [17]. Serum RBP4 might have clinical implications for lipid metabolism and insulin action [18]. However, to date, the clinical significance of RBP4 has been blunted by the study designs, which have tended to involve a heterogenous group composed of obese individuals and individuals with glucose intolerance or type 2 diabetes mellitus. Unlike these previous studies, we enrolled a homogenous group of women who possessed similar health characteristics.

In this study, we confirmed that serum RBP4 concentrations in women at least 50 years of age were higher than those in women younger than 50 years. There are several possible explanations. As women older than 50 years reach menopause, estrogen decreases. As such, fat amounts or body fat percentages change; and visceral fat increases. As a result, lipid metabolism becomes dysregulated. This change of lipid metabolism may affect serum RBP4 concentrations that come from adipocytes [18]. In addition, RBP4 concentrations are sex dimorphic, with different fat amounts and the influences of sex hormones producing higher concentrations in men than in women [20,21]. As stated above, the differences in our study are attributed to the decreased sex hormone status. However, these differences stem not only from a decreased estrogen status but also because of the relative changes in testosterone and other androgens.

Age is an independent risk factor for IR [11,12]. Many factors such as overall increased adipose tissue and decreased physical activity predispose older people to develop glucose intolerance and IR. In our study, although there was no significant difference in fasting glucose and HOMA-IR between women at least 50 years of age and women younger than 50 years, serum RBP4 concentrations in women at least 50 years of age were significantly higher than those in women younger than 50 years. Retinol-binding protein 4 positively correlated with age and HOMA-IR in women at least 50 years of age. After multiple linear regression analysis, serum RBP4 concentrations were still associated with HOMA-IR in women at least 50 years of age but not in women younger than 50 years. This suggests that serum RBP4 levels may reflect age-induced IR.

Presently, serum RBP4 concentrations were positively correlated with age, diastolic pressure, fasting glucose, and HOMA-IR in all subjects. These variables are linked to IR, cardiovascular disease risk factors, and metabolic syndrome. After subgroup analysis, serum RBP4 concentrations in women at least 50 years of age correlated with age, fasting glucose, and HOMA-IR. In addition, serum RBP4 concentrations correlated with only fasting glucose in women younger than 50 years. That means serum RBP4 concentrations may be predictive of IR in women at least 50 years of age but not in women younger than 50 years. As described earlier, women older than 50 years can be influenced by many changes that affect factors such as hormone concentrations, fat amount, and lipid metabolism. The relationship between RBP4 and gonadotropin may also contribute to the changes in insulin sensitivity associated with menopause [22]. These diverse factors may affect IR in women at least 50 years of age. This vulnerable condition to IR in women of this age explains our observations of the independent correlations of HOMA-IR with serum RBP4 concentrations after multiple regression. However, the β-coefficient for fasting insulin was negative (Table 5). We do not believe that this indicates an inverse relationship between fasting insulin and RBP4 concentrations. Rather, it is our belief that, after multiple linear regression analysis, the independent degree of strength of fasting insulin became weaker than before analysis.

We had intended to find the association between RBP4 and smoking, which is a cardiovascular risk factor. However, the study group did not include any smokers. Indeed, the prevalence of smoking in South Korean women in a 2008 survey was only 4.1% [23].

Fasting glucose positively correlated with serum RBP4 concentrations in both women younger than 50 years and women at least 50 years of age. After simple linear regression analysis, fasting glucose was still associated with serum RBP4 concentrations in all subjects and 2 age groups. Obesity but not hyperglycemia is an important determination of serum RBP4 concentrations [15]. However, the association between serum RBP4 concentrations and fasting glucose may be explained by the mechanism through which RBP4 develops IR in liver. Retinol-binding protein 4 induces the expression of the gluconeogenic enzyme phosphoenolpyruvate carboxykinase in the liver [15]. The present results could be due to this mechanism.

Body fat, waist size, waist-hip ratio (WHR), and BMI had inverse relationship with serum RBP4 in women younger than 50 years. Compared with women older than 50 years, women younger than 50 years have higher value of BMI but have lower value of waist size and WHR. It is possible that they have lesser visceral fat amount despite higher value of BMI than normal. However, it is not clear why they have inverse relationship; and it needs more study.

This study has a few limitations. First, we could not reflect each individual menopausal state accurately. We had difficulty in ascertaining the menopausal records of the subjects, so we arbitrarily but reasonably divided women based on a published benchmark age of 50 years. Second is the small sample size. Large-scale studies will be necessary to identify associations between serum RBP4 concentrations and menopause. Third, we could not find an association between smoking and serum RBP4 concentrations; so there is a need for more studies that include smoking subjects. Finally, HOMA-IR is a less precise approach compared with euglycemic clamp technique to assess IR [24,25]. Clamp studies are actually the criterion standard for analyzing IR, but we used HOMA-IR for convenience.

In conclusion, serum RBP4 concentrations are higher in women at least 50 years of age than in those younger than 50 years; and serum RBP4 is independently associated with IR in women at least 50 years of age. We could not clearly determine why serum RBP4 concentrations increase with age, but the data are consistent with the suggestion that age-induced IR may be reflected by serum RBP4 concentrations. In addition, the hepatic gluconeogenic mechanism of RBP4 is an important mechanism to study to determine the association between RBP4 and IGT. However, it has yet to be determined whether an RBP4 change under physiologic conditions is due to menopause or age. The clinical implications of RBP4 for predicting cardiovascular diseases and metabolic syndrome need further investigation.

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