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Relationship between serum resistin concentrations and inflammatory markers in patients with type 2 diabetes mellitus

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Abstract

To examine whether serum resistin concentrations are associated with metabolic or inflammatory markers in patients with type 2 diabetes mellitus, we examined serum concentrations levels and metabolic or inflammatory markers in 56 patients with type 2 diabetes mellitus and 41 healthy subjects. Serum levels of resistin, serum amyloid A, and soluble vascular cell adhesion molecule-1 were measured by enzyme-linked immunosorbent assay. Serum resistin levels were significantly elevated in diabetic patients compared with those in healthy subjects. Serum resistin concentrations did not correlate with body mass index; however, there was a significant positive correlation between resistin and soluble vascular cell adhesion molecule-1 in diabetic patients. Based on the present results, we conclude that resistin appears to be associated with vascular inflammatory markers in patients with type 2 diabetes mellitus.

1. Introduction

Recent studies have indicated that adipose tissue is not only an organ for energy storage but also an endocrine organ that produces a number of bioactive proteins referred to as adipocytokines [1,2]. Resistin is a recently identified adipocytokine [3]. Initial studies of rodents have indicated that resistin is expressed in adipose tissue, and its expression is up-regulated in animal models of obesity [4]. However, in human adipose tissue, resistin is detectable only at very low levels to date, and no relationship between resistin expression and obesity has been reported [5,6]. Therefore, it appears that resistin may be regulated by different mechanisms in humans and rodents [7]. Furthermore, the expression of resistin in adipocytes has not been shown to differ among healthy or insulin-resistant individuals [8].

Although it remains unclear whether resistin plays a role in the link between human obesity and type 2 diabetes mellitus, this protein is detectable in peripheral blood monocytes and it may play a role in inflammatory processes as well [9]. The expression of resistin in human monocytes has been shown to be induced by treatment with proinflammatory cytokines [10]. Furthermore, resistin has been shown to up-regulate the expression of adhesion molecules in human endothelial cells, thus suggesting its potential role in atherosclerosis [11]. However, the relationship between resistin and inflammation or atherosclerosis in human diseases remains largely unexplored. In this study, we examined whether serum levels of resistin are associated with inflammatory markers in patients with type 2 diabetes mellitus.

2. Patients and methods

2.1. Patients

Serum samples were taken from 56 patients with type 2 diabetes mellitus who fulfilled the World Health Organization criteria for diabetes mellitus and attended the Nagasaki Medical Center diabetes clinic. Of these patients, those who were receiving either thiazolidinedione or metformin therapy were excluded from this study. The body mass index (BMI) was calculated as weight (kilograms) divided by height squared (square meters).

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Forty-one healthy subjects (11 men and 30 women) with a mean BMI of 21.9 kg/m^2 were recruited as controls for this study. Healthy subjects were defined as individuals with a blood pressure of less than 140/90 mm Hg, normal renal and liver function, a fasting plasma glucose level of less than 90 mg/dL, and a postprandial 2-hour plasma glucose level of less than 140 mg/dL. None of the subjects in the healthy control group had other diseases.

2.2. Collection and analysis of serum samples

After the subjects underwent a 12-hour fast, blood samples were collected for the determination of serum glucose, insulin, resistin, glycosylated hemoglobin (HbA_{1c}), soluble vascular cell adhesion molecule-1 (sVCAM-1), serum amyloid A (SAA), and interleukin 6 (IL-6). All anthropometric data were collected at the same time as the blood sample collection.

2.3. Laboratory analysis

Total cholesterol, triglyceride, and low-density lipoprotein (LDL) cholesterol levels were measured in the serum according to automated enzymatic procedures (TBA200FR, Toshiba, Tokyo, Japan). HbA_{1c} was measured with an HA-8160 analyzer (Arklay, Kyoto, Japan).

2.4. Enzyme-linked immunosorbent assay

Serum resistin was measured in duplicate by using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Bio Vender, Brno, Czech Republic). The antibodies used in this ELISA kit have no detectable cross-reactivity to mouse resistin or other cytokines in human serum. Serum levels of sVCAM-1 and IL-6 were determined by using an ELISA kit (R&D Systems, Minneapolis, MN) according to the manufacturer's recommendations. Serum levels of SAA were determined by hSAA ELISA kit (Biosource, Camarillo, CA).

2.5. Statistical analysis

All data are presented as mean ± SD. The statistical analysis was performed with StatView (SAS Institute, Cary, NC) for Windows version 6.0. Resistin was log-transformed to normalize distribution. Any differences between the 2 groups were evaluated by the Mann-Whitney *U* test for nonparametric variables. Simple correlations were assessed by using Pearson correlation coefficient test. Multiple regression analysis was performed to analyze the correlations between resistin and clinical or laboratory data. The threshold for significance was a *P* value less than .05.

Table 1
Clinical and metabolic characteristics of patients with type 2 diabetes mellitus

	Mean ± SD	Minimum	Maximum
Sex (male/female)	27/29		
Age (y)	59.2 ± 11.9	16	84
BMI (kg/m^2)	24.1 ± 4.6	17.4	37.9
Systolic blood pressure (mm Hg)	134 ± 19	100	180
Diastolic blood pressure (mm Hg)	77 ± 12	50	104
Total cholesterol (mg/dL)	193.1 ± 40.5	121	317
Triglycerides (mg/dL)	138.9 ± 80.9	41	377
LDL cholesterol (mg/dL)	117.8 ± 35.0	55	205
HbA_{1c} (%)	7.81 ± 1.81	5.2	14.7
Resistin (ng/mL)	5.41 ± 4.35	1.32	25.87
SAA (μg/mL)	9.89 ± 9.22	0.59	42.16

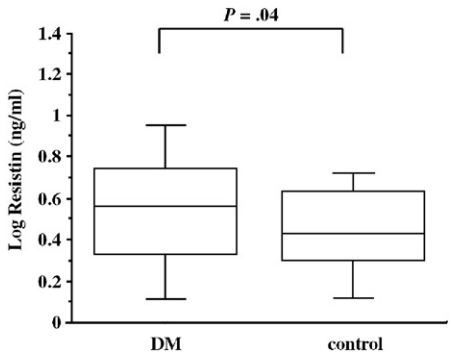

Fig. 1. Serum resistin levels in patients with type 2 diabetes mellitus and healthy subjects. The box contains the values between the 25th and 75th percentiles and the horizontal line is the median. The error bars stretch the 10th to 90th percentiles. Serum resistin is significantly greater in the diabetic patients than in healthy subjects.

3. Results

Table 1 summarizes the anthropometic and metabolic parameters of 56 patients with type 2 diabetes mellitus in this study. The control subjects were slightly younger than the diabetic patients enrolled in the study (control subjects, 49.4 ± 16.2 years; diabetic patients, 59.2 ± 11.9 years). The BMI appeared to be higher in diabetic patients than in healthy subjects; however, no statistically significant difference was observed (control subjects, 21.9 ± 3.3; diabetic patients, 24.1 ± 4.6). Comparison of fasting resistin in type 2 diabetic patients and healthy subjects revealed that the diabetic patients had higher resistin levels than the healthy subjects (diabetic subjects, 5.41 ± 4.35 ng/mL; healthy subjects, 3.18 ± 1.61 ng/mL; Fig. 1).

Table 2
Simple correlation analysis between serum resistin and clinical and laboratory data

	Coefficient	*P*
BMI	−0.17	.907
Age	0.094	.496
HbA_{1c}	0.172	.109
Log total cholesterol	0.279	.079
Log triglycerides	0.149	.284
Log LDL cholesterol	0.386	.056
Log SAA	0.406	.014
Log sVCAM-I	0.470	.004
Log IL-6	0.470	.002

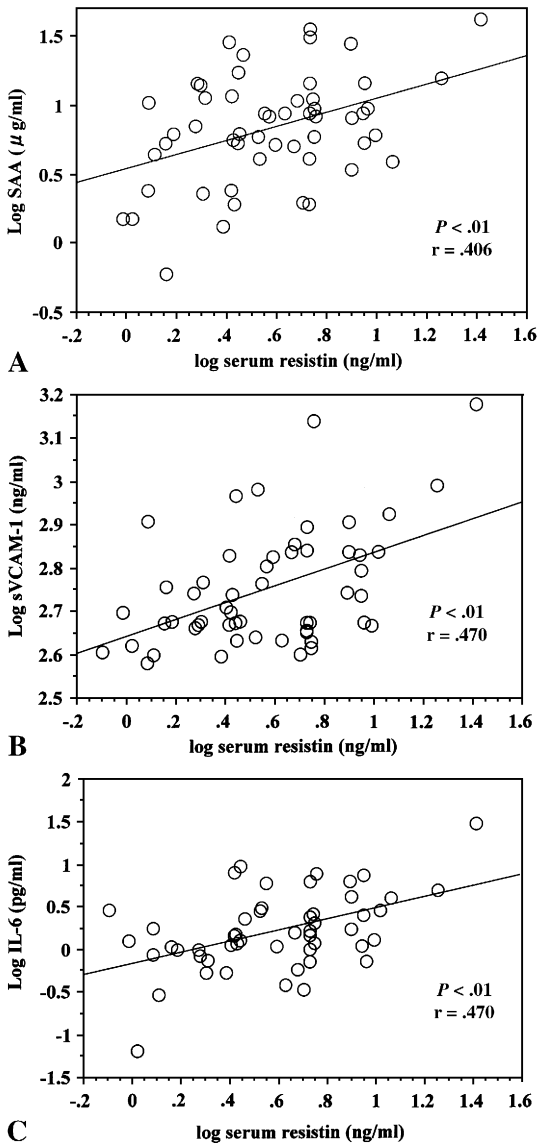

Fig. 2. Correlations between serum resistin concentrations and SAA (A), sVCAM-1 (B), or IL-6 (C) in patients with type 2 diabetes mellitus. There are positive correlations between serum resistin concentrations and SAA, sVCAM-1, or IL-6 in diabetic patients.

Adipocytokines are known to play various roles in the development of atherosclerosis [12]. Therefore, we investigated whether resistin is linked to vascular inflammatory markers, such as SAA and sVCAM-1, which are known to be reflective vascular proinflammatory states, and thus are predictive of a risk for cardiovascular diseases. Results of simple correlation analysis with clinical and laboratory data from patients with type 2 diabetes mellitus are listed in Table 2. Serum resistin correlated significantly with SAA, IL-6, and sVCAM-1 (Fig. 2A-C), but not with clinical parameters such as BMI, HbA_{1c}, total cholesterol, and triglyceride. Results of multiple regression analysis are listed in Table 3. After adjustment for potential confounding factors, only sVCAM-1 was independently related to serum resistin concentrations.

4. Discussion

Adipose tissue secretes several factors that modulate insulin resistance such as leptin and adiponectin [2]. Resistin was initially discovered in mice, in which it is predominantly expressed in adipocytes [4]. In contrast, macrophages, rather than adipocytes, appear to the most important source of resistin in human subjects [9]. These observations suggest that resistin is linked to inflammation as well as to insulin resistance. In our study, serum resistin concentrations were significantly elevated in patients with type 2 diabetes mellitus compared with those of healthy controls. These results are in good agreement with those of a study by McTernan and coworkers [13] that showed higher levels of serum resistin in type 2 diabetic subjects compared with those of nondiabetic subjects. Although possible associations between circulating resistin and various metabolic parameters were investigated in the present study, serum resistin concentrations were not found to be related to any of these indices.

Recent findings have indicated that the stimulation of macrophages in vitro with proinflammatory cytokines leads to a marked increase in resistin production [14]. Lehrke et al [15] demonstrated that endotoxemia caused a dramatic increase in resistin and it was hypothesized that tumor necrosis factor α is responsible for the induction of resistin. These data suggest that hyperresistinemia could be associated with systemic inflammation via tumor necrosis factor α.

Thus, in human subjects, resistin appears to be induced during the inflammatory response. Therefore, we investigated whether serum resistin concentrations were associated with these inflammatory markers in patients with type 2 diabetes mellitus. We have demonstrated a correlation between resistin and inflammatory markers, such as SAA, IL-6, or sVCAM-1. However, the correlation rate between resistin and these inflammatory markers was relatively low. This weak correlation could be explained by the idea that elevated levels of SAA or IL-6 could be an epiphenomenon of the subclinical inflammation. On the other hand, only sVCAM-1 was independently correlated with serum resistin in our multiple regression analysis. Further studies are required to confirm the role of resistin and the relationship between resistin and these inflammatory markers in patients with diabetes.

Table 3
Multiple regression analysis for independent determinants of serum resistin

	Coefficient	*P*
BMI	0.17	.920
Age	0.095	.498
HbA_{1c}	0.245	.092
Log total cholesterol	0.041	.888
Log triglycerides	0.198	.333
Log LDL cholesterol	0.334	.215
Log SAA	0.125	.419
Log sVCAM-I	0.444	.020
Log IL-6	0.129	.480

Obesity and type 2 diabetes mellitus are thought to be associated with the activation of innate immune pathways and chronic inflammation [16]. Furthermore, inflammatory processes are known to be involved in the development and progression of atherosclerosis and its complications [17]. More recently, Reilly and colleagues [18] demonstrated that resistin was associated with C-reactive protein in nondiabetic patients as well as in patients with type 2 diabetes mellitus, and resistin was also found to be associated with coronary artery calcification, a quantitative index of atherosclerosis. Verma and colleagues [19] also demonstrated that resistin up-regulated VCAM-1 and endothelin expression in human endothelial cells. During endothelial activation, shed forms of cell adhesion molecules (CAMs) are detected in circulation [20]. Increased levels of CAMs, such as sVCAM-1, have been detected in patients with cardiovascular disease and type 2 diabetes mellitus [21]. With regard to the observed positive correlation between the concentrations of resistin and sVCAM-1, it is possible that resistin is likely to be involved in low-grade vascular inflammation in patients with type 2 diabetes mellitus.

In conclusion, serum resistin levels were found to be increased in patients with type 2 diabetes mellitus. Resistin was also associated with a vascular inflammatory marker, sVCAM-1, suggesting that resistin may be related to subclinical vascular inflammation in diabetes. Further studies will be needed to identify a relationship between resistin and cardiovascular complications in patients with type 2 diabetes mellitus.

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