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The serum levels of resistin in rheumatoid arthritis patients

K. Migita[1,2], Y. Maeda[1], T. Miyashita[1,2], H. Kimura[2], M. Nakamura[1], H. Ishibashi[1], K. Eguchi[3]

[1]Clinical Research Center and [2]Department of General Internal Medicine, NHO Nagasaki Medical Center, Kubara 2-1001-1, Omura 856-8652, Japan; [3]First Department of Internal Medicine, Nagasaki University School of Medicine Sakamoto 1-7-1, Nagasaki 852-8501, Japan.

Kiyoshi Migita, MD; Yumi Maeda, PhD; Taichiro Miyashita, MD; Hironori Kimura, MD; Minoru Nakamura, MD; Hiromi Ishibashi, MD; Katsumi Eguchi, MD.

Please address correspondence to: Kiyoshi Migita MD, Clinical Research Center, NHO Nagasaki Medical Center, Kubara 2-1001-1, Omura 856-8652, Japan.
E-mail: migita@nmc.hosp.go.jp

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ABSTRACT

Objective. *Adipocyte-derived resistin is a circulating protein implicated in insulin resistance, but the role of human resistin is uncertain because it is produced largely by macrophages. The aim of this study was to analyze serum resistin concentrations in rheumatoid arthritis (RA) patients to determine the role of resistin in human inflammatory diseases.*

Materials and methods. *Resistin concentrations were assessed by ELISA in serum samples from 42 patients with RA. Serum samples from 38 healthy subjects acted as controls. We also evaluated the circulating levels of tumor necrosis factor-α (TNF-α) and disease activity markers in RA patients.*

Results. *In RA patients, serum resistin levels were significantly higher than those in healthy subjects. Serum resistin levels in RA patients were correlated with the RA disease activity markers, CRP and ESR. Furthermore, resistin levels in RA patients were significantly correlated with circulating levels of TNF-α.*

Conclusion. *Serum resistin levels were significantly increased in RA patients and correlated with inflammatory markers and TNF-α, suggesting that resistin may play a role in the rheumatoid inflammatory process.*

Introduction

Adipocytes secrete several active molecules such as leptin, adiponectin and resistin (1).

These substances, collectively known as adipokines, may function as signaling molecules that influence insulin sensitivity (2). These products secreted by adipose tissues also play a role in inflammation and immune responses (3). Resistin was initially described as an adipocyte-derived mediator of hepatic insulin resistance and is regulated by peroxisome proliferator-activated receptor gamma (4). Initial studies in rodents suggest that resistin is upregulated in obesity and may be involved in the development of insulin resistance (5). Later studies have failed to confirm this hypothesis and demonstrated reduced resistin expression in human adipose tissues (6). Although resistin can be detected at very low levels in human adipose tissues, this protein is also detectable in peripheral blood mononuclear cells suggesting its possible role in inflammatory processes (7). Therefore, resistin is thought to be linked to inflammatory diseases as well as metabolic diseases. Because inflammatory cytokine production is involved in the pathogenesis of rheumatoid arthritis (8), we investigated whether circulating levels of resistin are elevated in patients with rheumatoid arthritis (RA).

In the present study, we found that serum levels of resistin were significantly elevated in RA patients compared to those of healthy controls, and that the levels were correlated with serum levels of tumor necrosis factor (TNF)-α in RA patients. Our results suggest that resistin could be modulated by inflammatory cytokines and may play a role in the inflammatory process of RA.

Patients and methods

Patients

Serum samples were collected from 42 RA patients who fulfilled American College of Rheumatology classification criteria for RA, and who attended the Rheumatology Clinic of Nagasaki Medical center. RA patients with metabolic diseases, infections and other inflammatory diseases were excluded in this study. The collected samples were stored at -70ºC until use. Thirty eight healthy subjects (7 men and 31 women) with a mean body mass index (BMI) of 20.6 kg/m^2 were recruited as controls for this study (Table I). Healthy subjects were defined as individuals with a blood pressure of less than 140/90 mmHg, 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. No healthy subjects of them were suffering from other diseases. Informed consent was obtained from each individual.

Laboratory analysis

Serum levels of C-reactive protein (CRP) were measured by standard nephelometry, with established normal

Table I. Clinical characteristics of RA patients.

	RA patients (n = 42)	healthy controls (n = 38)
Age, years (range)	53.3 ± 15.4 (31-82)	45.5 ± 13.3 (28-76)
Gender, male / female	10/32	7/31
Disease duration, years (range)	7.0 ± 6.5 (1-40)	NA
DAS28 (CRP)	3.6 ± 1.0	NA
BMI	22.5 ± 4.4	20.6 ± 2.3
Treatment		
Steroid	30/42	
Methotrexate	9/42	
Other DMARDs	35/42	

NA: Not analyzed; DMARDs: disease-modifying anti-rheumatic drugs.

range 0-3 mg/l. Erythrocyte sedimentation rates (ESR) was measured by the Westgren method with the normal range defined as 0-14 mm/hr. Total cholesterol, triglyceride, and LDL cholesterol levels were measured in serum automated enzymatic procedures (TBA200FR, Toshiba, Japan). HbA1c was measured by HA-8160 analyzer (Arklay, Japan).

ELISA

Serum levels of TNF-α were determined by enzyme-link immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN, USA) following to the manufacturer's instructions. Serum levels of resistin were determined by sandwich ELISA (Bio Vendor, Brno, Czech Republic).

Statistical methods

The levels of continuous variable were expressed as mean ± SEM. Differences between groups were calculated using the Mann-Whitney U test. The level of correlation between resistin and biochemical characteristics was assessed by Pearson's correlation test. *P* values of < 0.05 were considered to be significant. All statistical analyses were performed with version 6.0 of StatView for Windows (SAS Institute Inc, Cary, NC, USA)

Results

Clinical data of the RA patients at presentation

Table I presents the clinical data of the RA patients and control subjects who were all of similar sex, height, body weight, BMI and age.

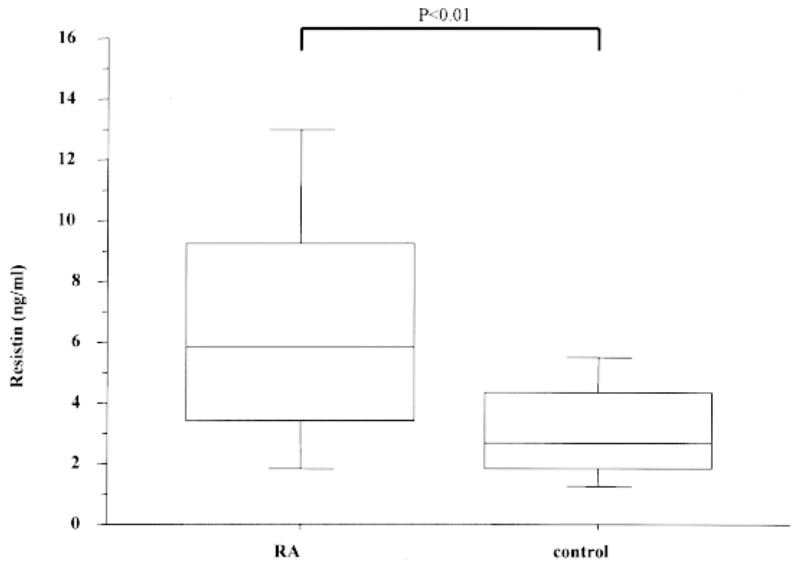

Fig. 1. Serum resistin levels in RA patients and healthy subjects.
The box contains the values between the 25th and 75th percentiles and the horizontal line is the median. The error bars stretch the 10th to 90th percentiles.

Increased levels of resistin in the blood of RA patients

As shown in Figure 1, serum resistin levels were found to be significantly higher in RA patients compared to those in control subjects (RA, 6.72 ± 4.59ng/ml; control subjects, 3.15 ± 1.66ng/ml; P = 0.0005). The correlations between resistin and metabolic or inflammatory markers in RA patients are listed in Table II. There was a significant positive correlation between resistin and inflammatory markers (CRP, ESR). On the other hand, there was no correlation between resistin and BMI or dose of prednisolone (Table II).

Correlations between serum levels of resistin and cytokines in RA patients

We sought to evaluate the relationship between resistin and an inflammatory cytokine, TNF-α, which is thought to be key pathogenic factors in RA. As reported previously (8), serum levels of TNF-α (Fig. 2A) were significantly higher in RA patients than in healthy subjects. The correlation between serum levels of resistin and circulating TNF-α reached statistically significant levels (Fig. 2B).

Discussion

Resistin (FIZZ3 / ADSF) is an adipocyte-derived peptide first identified during a search for targets of thiazolidinediones (4). Steppan *et al.* (5) reported that serum concentrations of resistin were markedly increased in obese mice and decreased by treatment with thiazolidinediones. Therefore, resistin has been thought to be linked in obesity with insulin resistance and diabetes in a mouse model (9). However, in humans, the expression of resistin in adipocytes is very low compared with that in rodents, but resistin mRNA is detectable in circulating mononuclear cells (7), which suggests that human resistin plays a role in inflammatory conditions.

The present study showed significantly increased resistin levels in the sera of RA patients compared with healthy subjects. Furthermore, the increased serum levels of resistin correlated with CRP or ESR in RA patients. This positive correlation between resistin and

inflammatory markers in RA patients suggests that the increased resistin values found in RA patients could be linked to the rheumatoid inflammation. Our data differ from those presented in a recent study by Bokarewa *et al.* (10) in which blood resistin levels were not significantly different between RA patients and controls. Although the reason for this discrepancy is unknown, blood resistin levels were relatively lower in our control subjects than in the controls in Bokarewa's study. Serum resistin levels in our control subjects, however, were quite similar to those reported data by Heilfronn (11), in whose study circulating resistin levels of non-obese and non-diabetic healthy subjects were analyzed by the same ELISA kit used in the present study.

There was a significant positive correlation between serum resistin and TNF-α in our study. Lehrke *et al.* showed that lipopolysaccharide (LPS), a potent inflammatory stimulant, increases resistin production by inducing the secretion of the inflammatory cytokine TNF-α in humans (12). These data suggest that hyperresistinemia could be associated with systemic inflammation via TNF-α, as well as metabolic disorders such as obesity or insulin resistance. The increased circulating resistin levels of the RA patients in the present study could have been induced by rheumatoid inflammation via the inflammatory cytokine, TNF-α. Bokarewa *et al.* recently demonstrated that resistin displays proinflammatory properties by strongly up-regulating IL-6 and TNF-α from human PBMCs (10). Alternatively, the positive correlation between serum levels of resistin and TNF-α in RA patients suggests that resistin partly contributes to the inflammatory cytokine production in these patients.

More recently, Otero *et al.* reported that there was no significant difference in serum resistin levels between RA patients and healthy subjects (13). The reason for this discrepancy could be differential characteristic of the investigated RA patients. In our study, no RA patient was treated by TNF-blocker. In contrast, 26% of RA patients were treated by TNF antagonists, and mean CRP was controlled to relatively low levels in their study (mean CRP = 0.61 ± 0.19mg/dl; vs. our RA patients = 2.47 ± 0.79mg/dl). Recent report indicated that hyperresistinemia is linked with elevation of TNF-α in a human study (12). Therefore, it is possible that serum resisting levels could be influenced by RA disease activity in RA patients.

RA patients appear to have a higher risk of cardiovascular disease (CVD)

Table II. Correlations among resistin and metabolic or inflammatory markers in RA patients.

	Resistin	BMI	Total cholesterol	Triglycerides	ESR	CRP
Resistin						
BMI	-0.056					
Total cholesterol	-0.027	0.425**				
Triglycerides	0.048	0.430**	0.445**			
ESR	0.430**	-0.226	-0.021	-0.079		
CRP	0.390*	-0.145	-0.138	0.077	0.656**	
Dose of PSL	0.039	0.157	0.079	0.441*	0.540**	0.673**

BMI: body mass index; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; PSL: prednisolone; *P< 0.05; **P < 0.01.

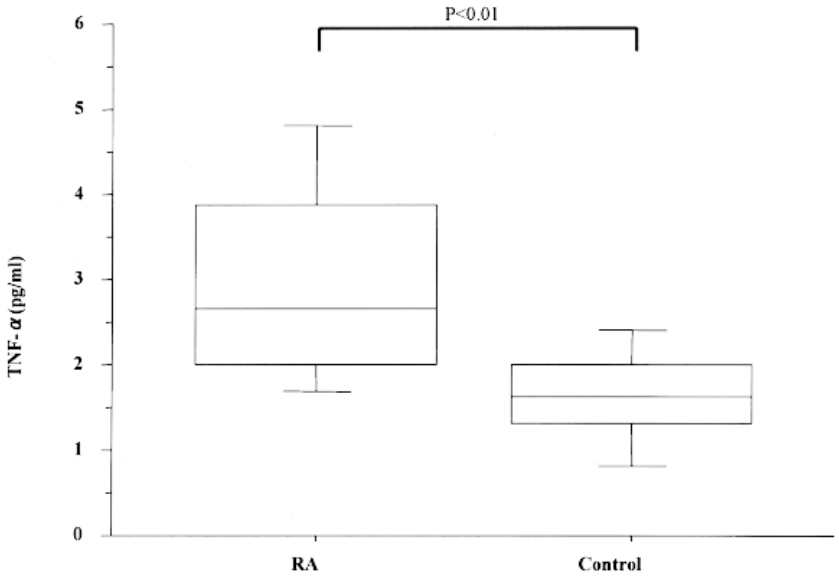

Fig. 2A Serum TNF-α levels in RA patients and healthy subjects.
The box contains the values between the 25th and 75th percentiles and the horizontal line is the median. The error bars stretch the 10th to 90th percentiles.

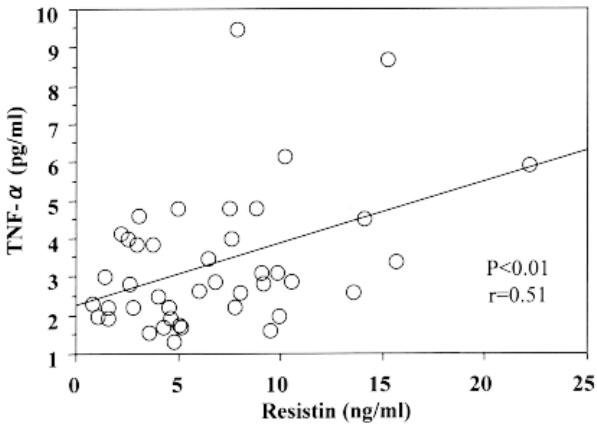

Fig. 2B Correlation between serum levels of resistin and TNF-α in RA patients.
TNF-α: tumor necrosis factor-α.

and mortality (14). Previous evidence supporting an inflammatory basis for atherosclerosis suggests a link between rheumatoid inflammation and the risk of CVD (15). Recent study indicated that circulating resistin levels are correlated with vascular inflammatory markers and coronary atherosclerosis in humans (16). These findings suggest that in addition to inflammatory cytokines, resistin may also be involved in the inflammation-based etiology of atherosclerosis in RA.

In conclusion, our study demonstrates a significant increase in circulating resistin levels in patients with RA compared with healthy controls. These increased levels of resistin may be linked with the chronic inflammation of RA patients, and resistin is a candidate for inflammatory cytokines contributing to the rheumatoid inflammatory process.

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