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Increased serum resistin levels in patients with type 2 diabetes are not linked with markers of insulin resistance and adiposity

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Abstract The role of resistin in human biology remains uncertain. We measured serum resistin levels in Japanese patients with (n=111) and without (n=98) type 2 diabetes mellitus and investigated the significance of this hormone in the pathophysiology of diabetes. The levels of serum adiponectin and leptin were also measured. Resistin levels were increased significantly in patients with type 2 diabetes compared with non-diabetic subjects (24.7±2.6 *vs.* 15.0±1.2 ng/ml, p=0.0013). However, there was no correlation in either patient group between serum resistin levels and markers of insulin resistance, obesity or hyperlipidaemia. These results were in direct contrast to the data of leptin or adiponectin, both of which were closely related to these clinical markers of diabetes. Multivariate regression analysis on the combined data of the two groups demonstrated that the presence of diabetes and HDL cholesterol levels were significant predictors of serum resistin levels (diabetes: β=0.159, p=0.035; HDL: β=-0.172, p=0.039). No correlation was observed between C-reactive protein and resistin adjusted for BMI. Taken together, these findings demonstrate that serum resistin levels are increased in patients with type 2 diabetes, but this increase is not linked to markers of insulin resistance or adiposity. Further studies are necessary to elucidate the significance of serum resistin concentration in human pathophysiology.

Key words Resistin • Insulin resistance • Type 2 diabetes • Human

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Introduction

Resistin is a novel hormone secreted by adipocytes and belongs to the family of cysteine-rich C-terminal proteins known as RELM (resistin-like molecules) or FIZZ (found in inflammatory zone) [1–3]. The hormone was isolated initially as an mRNA, the expression of which was subsequently shown to be suppressed by peroxisome proliferator-activated receptor-γ agonists. Studies in rodent models of diabetes demonstrated that these agents enhance insulin sensitivity [1], suggesting resistin may have a causative role in insulin resistance [1, 4, 5]. Several human studies have shown, however, that resistin is unlikely to be an important factor linking human obesity to insulin resistance because of its low expression in human adipocytes [6–8]. Notwithstanding this fact, resistin protein is abundantly present in circulating human monocytes and could possibly be released from these cells into the serum [6, 7].

Resistin is present in the circulation and over the last few years several studies have investigated the pathophysiological significance of changes in circulating resistin levels. Although initial studies in rodents [1] and the one human study by Silha et al. [9] suggested a potential link of circulating resistin levels and insulin resistance, more recent studies demonstrated that resistin levels were not related to markers of insulin resistance and adiposity in humans [10–15]. In order to clarify these relationships there is a need for more data from different ethnic groups using different resistin assay systems.

We developed an ELISA for human resistin [16], and used it in this study to measure serum resistin levels in Japanese patients with type 2 diabetes. The data were then analysed to determine the relationships between resistin and other clinical markers of diabetes. Changes in resistin levels were also compared with changes in serum adiponectin and leptin levels, as the physiologic roles of these other two cytokines are well established.

Materials and methods

Subjects

One hundred and eleven patients with type 2 diabetes under 75 years of age were recruited from out-patient clinics of the Kyoto Prefectural University Hospital. Patients with malignancy, renal failure, severe liver injury, inflammatory disease or using either insulin, biguanide, thiazolidinedione or antihyperlipidaemic agents were excluded from the study. The control population consisted of 98 non-diabetic subjects (fasting blood glucose level <6.1 mmol/l and HbA1c level <5.8%) under 75 years of age who had visited a medical centre for a routine health check. The study protocol was approved by the Kyoto Prefectural University of Medicine institutional review board, and informed consent was obtained from each patient prior to participation in the study.

Collection and analysis of serum samples

Blood was obtained in the morning after an overnight fast for the determination of serum resistin, leptin, adiponectin, insulin, glucose, total cholesterol, HDL-cholesterol and triglyceride concentrations. At the same time, anthropometric measurements were obtained. The serum adiponectin, leptin and insulin levels were measured by RIA (Linco Research Inc., St. Charles, MO, USA), while serum resistin level was determined by ELISA as described previously [16]. Briefly, two types of polyclonal anti-human resistin antibody were produced against a synthetic peptide corresponding to residues 17–44 of human resistin and recombinant human resistin. Diluted samples were applied to a 96-well microtitre plate coated with purified anti-synthetic peptide IgG. The wells were washed and incubated further with biotinylated anti-recombinant human resistin IgG, followed by incubation with streptavidin-horseradish peroxidase. 3,3',5,5'-Tetramethylbenzidine and H_2O_2 were then added to each well and the absorbance read at 450 nm.

Measurement of C-reactive protein concentrations

A sub-group of age- and sex-matched subjects (20 males, 20 females) was selected from the diabetic and non-diabetic cohorts of the study. The serum level of C-reactive protein in these patients was measured using a commercially available ELISA kit (ANGIOPHARM, O'Fallon, MO, USA).

Statistical analysis

Data are expressed as mean±SEM. Analyses were performed using StatView 5.0 (SAS Institute Inc. Cary, NC, USA) with p values less than 0.05 being considered statistically significant. Differences between the two groups were assessed using Student's unpaired t-test while the Chi-square test was used for comparison of categorical variables. Logistic regression analysis was used to assess the association between diabetes and serum resistin levels after adjustment for confounding factors. Pearson's correlation coefficients were calculated followed by multivariate regression analysis using logarithm-transformed data of serum resistin, leptin, adiponectin, insulin, triglyceride and C-reactive protein levels and HOMA-R score.

Results

Clinical characteristics

The clinical characteristics of the non-diabetic and type 2 diabetic groups are summarised in Table 1. The diabetic group consisted of more males (p=0.0003) and was significantly older than the non-diabetic group (p=0.0006). In addition, serum triglyceride levels were higher (p=0.042) and serum HDL cholesterol levels lower (p=0.0013) in the patients with diabetes compared to the non-diabetic controls.

Serum resistin levels

As shown in Fig. 1, serum resistin levels were increased significantly in the patients with type 2 diabetes compared with non-diabetic subjects (24.7±2.6 *vs.* 15.0±1.2 ng/ml, p=0.0013). There was no difference in serum leptin levels between the two groups. The patients with type 2 diabetes had significantly lower serum adiponectin levels than the non-diabetic subjects (25.0±1.3 *vs.* 35.2±2.1 μg/ml, p<0.0001), a finding in accordance with previous reports [17].

Table 1 Clinical characteristics of the non-diabetic and diabetic subjects

	Non-diabetic controls	Diabetes	*p*
n	98	111	
Sex, male/female	42/56	75/36	0.0003, χ^2=14.8
Age, years	54.1±1.0	58.6±0.8	0.0006
BMI, kg/m^2	22.6±0.4	23.3±0.3	0.12
Fasting glucose, mmol/l	5.2±0.04	8.5±0.2	<0.0001
HbA1c, %	5.2±0.03	7.4±0.1	<0.0001
Fasting insulin, pmol/l	35±2	39±3	0.768
HOMA-R	1.4±0.1	2.5±0.2	<0.0001
Total cholesterol, mmol/l	5.65±0.08	5.62±0.08	0.817
Triglyceride, mmol/l	1.22±0.10	1.59±0.15	0.042
HDL cholesterol, mmol/l	1.50±0.04	1.35±0.03	0.0013
sBP, mmHg	124.6±1.7	126.1±1.8	0.534
dBP, mmHg	76.5±1.1	74.0±1.1	0.116
Duration of diabetes, years		9.4±0.8	
Treatment (diet/SU)		40/71	

HOMA-R, fasting insulin (μU/ml)xfasting glucose (mg/dl) divided by 405
Mean±SE

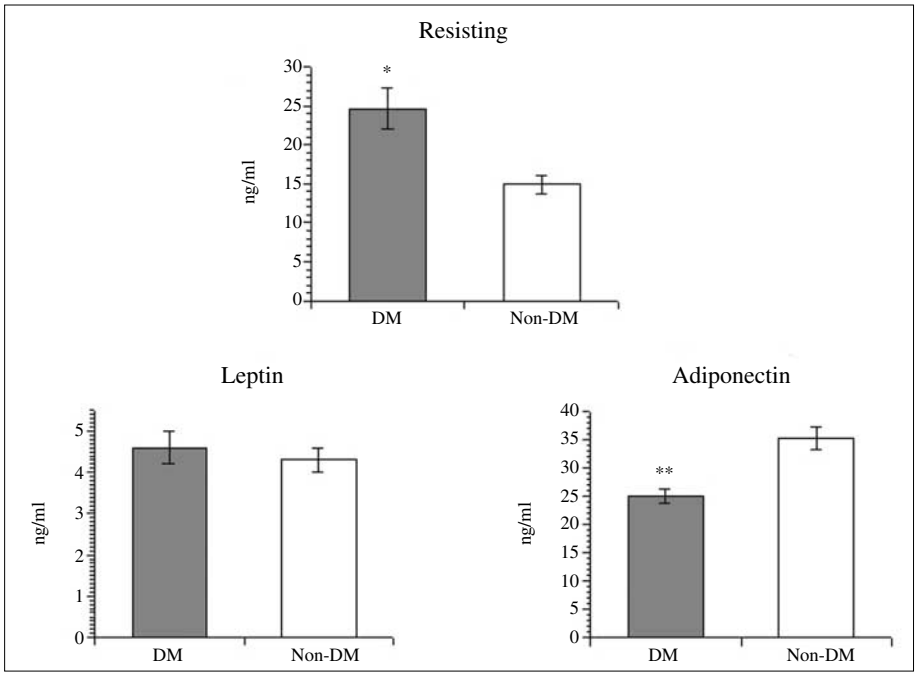

Fig. 1 Serum resistin, leptin and adiponectin concentrations in non-diabetic subjects (n=98) and patients with type 2 diabetes (n=111). *p=0.0013; **p<0.0001

Logistic regression analysis showed a significant association between serum resistin levels and the presence of type 2 diabetes after adjustment for gender, age, and triglyceride, HDL cholesterol, leptin and adiponectin levels (odds ratio 1.022 [95% CI 1.001–1.045], p=0.041).

There was no gender difference in serum resistin levels (non-diabetic: males 15.2±1.6, females 14.8±1.8 ng/ml, p=0.89; diabetic: males 21.8±2.3, females 30.8±6.3 ng/ml, p=0.10). In contrast, serum leptin and adiponectin levels were significantly higher in females compared with males in both the non-diabetic and diabetic groups (leptin: non-diabetic p<0.0001, diabetic p<0.0001; adiponectin: non-diabetic p=0.002, diabetic p=0.009).

Correlations between serum resistin levels and clinical markers

Pearson's correlation analyses were used to evaluate the relationship between serum resistin levels and clinical markers. In the non-diabetic subjects, no correlation was

found between serum resistin levels and other metabolic factors or markers of adiposity and insulin resistance. In the subjects with diabetes the only correlation observed was a weak association between serum resistin and insulin levels (r=0.21, p=0.032). In contrast, there was a significant positive correlation between serum leptin levels and insulin levels (r=0.62, p<0.0001), HOMA-R (r=0.60, p<0.0001) and BMI (r=0.65, p<0.0001), while serum adiponectin levels showed a significant negative correlation with these three parameters (insulin: r=-0.27, p=0.004; HOMA-R: r=-0.27, p=0.004; BMI: r=-0.40, p=0.0001). Accordingly, there was a significant negative correlation between leptin and adiponectin (r=-0.27, p=0.004) but no relationship between resistin and either leptin or adiponectin.

In order to determine the clinical factors that influenced the levels of resistin, leptin and adiponectin, we performed multivariate regression analysis using the combined data of the non-diabetic and diabetic groups. The independent variables used in the analysis were gender (score: males=0, females=1), BMI, presence of diabetes (score: without DM=0, with DM=1), HOMA-R, HDL cholesterol and triglyceride. Although the presence of diabetes and HDL were significant independent determinants of serum resistin levels (diabetes: β=0.159, p=0.035; HDL: β=-0.172, p=0.039), there was no relationship between resistin and either BMI or insulin. In contrast, gender (β=0.516, p<0.001), BMI (β=0.411, p<0.001), HOMA-R (β=0.324, p<0.001) and triglyceride level (β=0.101, p=0.035) were significant predictors of leptin, while gender (β=0.192, p=0.0028), BMI (β=-0.213, p=0.0018), presence of diabetes (β=-0.154, p=0.014), HDL cholesterol (β=0.181, p=0.0089) and triglyceride levels (β=-0.165, p=0.013) were significant predictors of adiponectin.

C-reactive protein and resistin

A recent clinical study suggested resistin may have a role in the sub-clinical inflammation that occurs commonly with diabetes [16], and therefore we investigated the relationship between serum resistin and the inflammatory marker, C-reactive protein. The levels of serum C-reactive protein were compared in age- (56.8±1.4 years) and sex- (20 males and 20 females) matched non-diabetic subjects (BMI 22.0±0.4, fasting insulin 32±2 pmol/l) and patients with type 2 diabetes (BMI 23.4±0.6, fasting insulin 41±4 pmol/l). Both serum resistin and C-reactive protein levels were significantly higher in the patients with diabetes compared with the non-diabetic controls (resistin 30.4±5.6 *vs.* 15.9±1.6 ng/ml, p=0.015; C-reactive protein 431.7±57.1 *vs.* 236.7±39.0 ng/ml, p=0.0061). Pearson's correlation analyses on the logarithmically transformed data revealed a weak but significant correlation between resistin and C-reactive protein in the patients with diabetes (r=0.32, p=0.043). This relationship was not significant after adjusting for BMI (r=0.146). No correlation was observed between resistin and C-reactive protein in the non-diabetic subjects (r=-0.187).

Discussion

This study demonstrated that serum resistin levels are significantly higher in Japanese patients with type 2 diabetes compared with non-diabetic subjects. This confirmed the results of our previous smaller study [16]. In addition, the present study clearly demonstrated that serum resistin levels did not correlate with any marker of insulin resistance, obesity or hyperlipidaemia, supporting the results of recent other studies [10–15]. Furthermore, we found no significant correlation between resistin and fasting blood glucose and HbA1c. These results were in direct contrast to our finding of a strong correlation between serum leptin and adiponectin levels and other clinical markers of diabetes, relationships that have been reported in previous studies. McTernan et al. [14] also showed that C-reactive protein was a significant predictor of serum resistin levels and suggested a potential role for resistin as a pro-inflammatory factor. However, in our study we found no correlation between C-reactive protein adjusted by BMI and resistin.

The reason for the increase in serum resistin levels in type 2 diabetes is unclear. It is possible that a more sensitive clinical marker of insulin resistance may correlate significantly with resistin, or alternatively serum resistin levels may be determined by some underlying factor associated with diabetes mellitus other than insulin resistance, serum glucose level or obesity.

It has been proposed that resistin may be a factor secreted by adipocytes that links insulin resistance and type 2 diabetes [18]. This proposal is based mainly on the results of *in vivo* studies in rodent models and investigations on the murine 3T3-L1 adipose cell line. Interpretation of these studies has, however, been complicated by contradictory findings on the expression and regulation of resistin [1, 4, 5, 19]. In contrast to rodent models, studies in humans have found low levels of resistin mRNA expression [6–8] and have also been unable to establish a clear link between resistin and insulin resistance. These findings suggest that the physiological and pathophysiological role of resistin in humans may be different from that in rodents. Evidence to support this possibility is that human and murine resistin have only 53% homology at the amino acid level [20] and that in humans the predominant site of resistin expression is monocytes with low expression in

adipocytes [6, 7]. Resistin belongs to the family of proteins named FIZZ [3], and is thought to act as a cytokine. Irrespective of resistin's possible role in insulin resistance, our finding of increased levels in diabetes provides more important information on the involvement of this molecule in human biology and diabetes, including vascular complications [21, 22].

Over the past few years several studies in humans have examined the relationship between circulating resistin levels and obesity or diabetes [9–15, 23]. The results of these studies have been difficult to interpret and contradictory as a consequence of differences in ethnicity and clinical background of the subjects investigated, or the target epitopes used in the resistin assays. Several studies investigating the relationship between circulating resistin and obesity have shown that levels are increased in obese subjects but are not associated with markers of insulin resistance or adiposity [11–13]. In contrast, Silha et al. [9] reported a significant correlation between resistin and HOMA-R. With regard to diabetes, a number of studies have described higher circulating resistin levels in diabetic compared with non-diabetic subjects, but that this increase was not associated with markers of insulin resistance or adiposity [10, 14, 15]. While the results of our study are in general agreement with these earlier studies, the reason for the lack of association between resistin and clinical markers of diabetes remains unanswered. These earlier studies also showed that plasma glucose [15] and C-reactive protein [14] were independent determinants of resistin levels, although we were unable to confirm either of these associations in our study. In contrast to these studies, an earlier study by Fehmann and Heyn did not show a significant difference between diabetic and non-diabetic subjects [23]. Taken together, these findings imply that secretion of resistin may be regulated by hormonal or inflammatory factors associated with obesity and diabetes, although the cellular origin of these factors remains unknown.

In conclusion, this study demonstrated an increase in fasting serum resistin levels in patients with type 2 diabetes that was not linked to markers of insulin resistance and adiposity. The implication of these findings are unclear and therefore further studies are required on the regulation, biological function and cellular source of human resistin in order to elucidate the diagnostic importance of increases in circulating resistin concentration.

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