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ORIGINAL ARTICLE

Serum Resistin Concentration in Obese Diabetic patients: Any Possible Relation to Insulin Resistance Indices?

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Resistin, an adipocyte secreted hormone, has been suggested to link obesity with type 2 diabetes and insulin resistance in rodent models, but its relevance to human diabetes remains uncertain. The aim of this study was to investigate the relationship between serum resistin concentrations with markers of insulin resistance and obesity in type 2 diabetes and non-diabetic obese subjects.

Materials and Methods: In this cross sectional study, consisting of 35 obese subjects with type 2 diabetes (16 women and 19 men, age 44.63±1.08 yr) and 35 obese non-diabetic subjects (19 women and 16 men, age 43.54±1.54 yr), fasting lipid profiles were measured by enzymatic methods; NycoCard HbA1c system was used to measure HbA1c. Serum resistin, insulin and glucose levels were measured by an enzyme immunoassay, and glucose oxidase methods respectively. Insulin resistance index was calculated from fasting glucose and insulin according to homeostasis model assessment (HOMA-IR).

Results: Mean insulin resistance index (HOMA-IR), HbA1c, diastolic blood pressure, triglycerides and fasting glucose in diabetics were significantly higher than in non-diabetic subjects ($p<0.05$). There was no significant difference in resistin levels between non-diabetic (7.16±3.72 ng/ml) and diabetic (6.40±3.66 ng/ml) obese subjects. Resistin levels in diabetic (7.46±3.98 vs. 5.51± 3.20 ng/mL) and non-diabetic (8.15±4.60 vs. 5.97±2.31 ng/mL) women were significantly higher than men in both groups. A significant negative correlation between diastolic blood pressure and resistin ($r= -0.381$; $p=0.024$) was observed only in the control groups.

Conclusion: Based on the results, it appears that resistin links between obesity and type II diabetes in humans is still a controversial topic and requires further investigation.

Key Words: Type 2 diabetes, Obesity, Resistin, HOMA-IR, BMI

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Introduction

Obesity and associated diabetes mellitus are epidemic through the world; obese individuals characteristically manifest with insulin resistance and hyperinsulinemia, which predispose to glucose intolerance, diabetes, and cardivascular disease.[1] Type 2 diabetes mellitus is a heterogeneous and polygenic disease associated with abnormal insulin secretion or defects of insulin action.[2,3] Insulin resistance, which implies a defect of insulin

signaling in the target tissues, is a common cause of type 2 diabetes and is related to obesity. The mechanisms underlying insulin resistance are not completely understood. However, adipose tissue plays an important role in insulin resistance through production and secretion of adipose-derived proteins "adipocytokines" such as tumor necrosis factor-α (TNF-α), plasminogen activator inhibitor-1 (PAI-1), leptin, angiotensinogen, adiponectin and resistin.[4-6]

Resistin, also called adipocyte-secreted factor (ADSF), found in inflammatory zone 3 (FIZZ3), is a novel hormone secreted by adipocytes; resistin has been implied to link obesity with insulin resistance and diabetes.[6,8,9] Resistin belongs to a family of cysteine-rich C-terminal proteins, termed RELMs (Resistin-Like Molecule), which include RELM-α / FIZZ 1, RELM-β / FIZZ 2 and a recently discovered RELM-γ, with each member having a unique differential tissue distribution.[7-10]

Initial studies in mice suggested that resistin mediates insulin resistance by antagonizing insulin action and modulating one or more steps in the insulin-signaling pathways.[6,11] However, conflicting animal data have since been presented, regarding whether in obesity resistin, production is increased[6,11] or decreased.[12-14] More importantly, the relevance and physiological role of resistin in humans remain relatively unknown. Given the incomplete homology (59%) between human and mouse resistin[9] and the absence in humans, of one of the three murine resistin isoforms, resistin in humans may have a different physiological role than that in mice. Moreover, several human studies have shown that resistin is unlikely to be an important factor linking human obesity to insulin resistance because of its low expression in human adipocytes.[15-17] In spite of this fact, resistin protein is abundantly present in circulating human monocytes and could possibly be released from these cells into the human serum.[15,16] Over the last few years several studies have investigated the pathophysiological significance of changes in circulating resistin levels. Although initial studies in rodents[6] and the one human study by Silha et al.[18] suggested a potential link of circulating resistin level and insulin resistance, more recent studies have demonstrated that resistin level is not related to markers of insulin resistance and adiposity in humans.[19-24] Thus, it seems that further studies are needed to understand the relationship between serum levels of resistin and insulin resistance in obese diabetic patients. The aim of this study was to investigate serum resistin level in type 2 diabetes and non-diabetic obese subjects and any relationship these levels may have with markers of insulin resistance and obesity.

Materials and Methods

Study patients

We studied 35 middle-aged obese individuals with type 2 diabetes (16 women and 19 men, age 44.63±1.08 yr, BMI: 34.89 ±0.67 kg/m2), who consecutively visited the out-patient clinics for diabetes mellitus, endocrine and metabolic diseases. Exclusion criteria included 1. Subjects with known diseases associated with disordered glucose metabolism 2. Subjects taking medications altering glucose metabolism 3. Subjects with chronic kidney disease 4. Smoking and 5. Alcoholism. In the present analysis, the American Diabetes Association criteria were used to diagnose diabetes mellitus, from casual capillary glucose levels.[25] Diabetic patients were treated with oral hypoglycemic agents (metformin, n=11, glibenclamide, n=5, and metformin plus glibenclamide, n=10); none received thiazolidinediones. The control group consisted of 35, middle-aged, non-diabetic obese individuals (19 women and 16 men, age 43.54±1.54 yr, BMI: 35.54±0.68 kg/m^2), who had undergone annual health check-ups. To select non-diabetic control individuals, the following inclusion criteria were used: 1. No diabetes in their first degree relatives. 2. Fasting serum glucose concentration less than 110 mg/dL. 3. Body mass index (BMI) ≥ 30. 4. Hemoglobin

$A1_C$ concentration less than 5.8%. 5. No smoking and 6. No alcoholism. Written informed consent was obtained from each subject and the institutional review board at Tabriz University of Medical Sciences approved the study protocol according to the Helsinki Declaration.

Anthropometric and blood pressure measurements

After obtaining written informed consent, the individuals were asked to complete questionnaires on anthropometric characteristics, general health, smoking, alcohol consumption, and present medications, including hormone replacement, oral hypoglycemic agents and oral contraceptive treatment. Obesity was defined as BMI≥30 kg/m^2. Anthropometric measurements were performed in all study participants before breakfast, with the subject wearing light clothing without shoes. For all subjects, weight and height were measured to the nearest 0.5 kg and 0.5 cm, respectively, and BMI was calculated as weight (kilograms) divided by height squared (square meters). Waist and hip circumference were measured with a soft tape in the standing position following normal expiration. Waist circumference was defined as the smallest girth midway between the lowest rib margin and the iliac crest. Hip circumference was measured at the level of the greater trochanter, all parameters being measured by well-trained dietitians. Waist to hip ratio (WHR) was calculated as waist circumference divided by hip circumference, anthropometric indices being measured by the same investigator so, as to minimize inter-observer variability measurements. Systolic and diastolic blood pressures were measured twice for each individual.

Blood Collection

Venous blood samples (5ml) were collected from all individuals after an overnight fast (≥ 10 h) between 8:30 and 9:30 AM. Blood was drawn from the antecubital vein. Sera, separated immediately after centrifugation with 3000 x g for 10 min, were stored at -70 °C until biochemical analyses were performed.

Biochemical Analysis

Fasting blood glucose concentration was measured by the glucose oxidase method (Pars Azmun. Tehran, Iran). Total cholesterol, triglycerides (TGs) and high-density lipoprotein-Cholesterol (HDL-C) were measured enzymatically using commercially available kits (Pars Azmun Co.Tehran, Iran). Low-density lipoprotein-Cholesterol (LDL-C) was estimated indirectly using the Friedwald formula for subjects with a serum TG concentration <400 mg/dL. Glycosylated hemoglobin was measured according to boronate affinity assay by NycoCard HbA1c System (Norway, with the coefficient of variation (CV) below 5%). Based on HbA1c percent, the diabetic group was sub grouped into the "well controlled" (n=24, patients with HbA1c below 8%) and "poorly controlled" (n=11, patients with HbA1c above 8%) subjects. Serum insulin concentration was measured by the Enzyme-Linked-Immuno Sorbent Assay (ELISA) using commercially available kits (Q1-DiaPlus, USA, Lot Number: 24Q1 L6). The sensitivity of the insulin assay was 0.5 µIU/mL. Intra- and inter-assay coefficients of variation were 6.45 and 6.45%, respectively.

Measurement of Resistin

Serum resistin was measured enzymatically using a commercially available kit, human resistin ELISA kit (BioVendor GmbH, Heidelberg, Germany; Cat.No:RD191016100, intra- and interassay coefficients of variation, 3.4 and 6.9 %, respectively). Before assay, sera were diluted 3-fold and, then 100µl of diluted sera, calibrator and quality control samples of human resistin were applied to 96-well micro titer plates. After incubation at room temperature for 60 min, the well was washed and incubated for 60 min; then following another washing step, it was incubated for 60 min with horseradish peroxidase conjugate. After the last washing step, the remaining conju-

gate was allowed to react the substrate H_2O_2-tetramethylbenzidine. Then, 100µL of acidic solution was added to each well to stop the reaction, and the absorbance at 450 nm was measured.

Homeostatasis Model Assessment

Since it plays a role in the development of atherosclerosis and diabetes mellitus, it is important to measure insulin resistance. Although the hyperinsulinemic euglycemic clamp[26] and steady-state plasma glucose value[27] are gold standards to estimate insulin resistance, they are used in complicated procedures. The Homeostasis Model Assessment (HOMA-R) is a convenient means to evaluate insulin resistance,[28] and was hence used; the HOMA model was used to assess pancreatic β-cell function (HOMA β-cell) , insulin resistance (HOMA-IR) and quantitative insulin sensitivity check index (QUICKI) using fasting insulin and glucose concentrations by the formula: HOMA β-cell=20× [fasting insulin (µIU/mL)÷[fasting glucose (mmol/L)–3.5], HOMA-IR = [fasting insulin (µIU/mL)×fasting glucose (mmol/L)÷22.5 and QUICKI=1/[log (glucose, mg/dL)+log (insulin, µIU/mL)].[29]

Statistical analysis

All continuous data are expressed as Mean±SD. Based on a pilot study, using an α value of 0.05 and a β value of 0.2 (80% power), the minimum sample size required was 32 samples per group. Statistics were analyzed using SPSS for windows version 14 software. Resistin levels of non-diabetic and diabetic group were compared using Student's t-test for independent samples. Correlations of resistin level with the other parameters were evaluated by Pearson correlation coefficient. For the comparison of the resistin levels among subgroups that had well and poorly controlled diabetes mellitus, the Student's unpaired t-test was used. For all statistical assessments, a value of $p<0.05$ was considered as statistically significant.

Results

Table 1 summarizes the anthropometric, metabolic and biochemical characteristics of non-diabetic and type 2 diabetes mellitus subjects. Serum resistin concentrations were not different between diabetic (6.40±0.61 ng/mL) and non-diabetic subjects (7.16±0.62 ng/mL). However, in sex adjusted analysis, the mean concentrations of serum resistin in diabetic (7.46±0.29 vs. 5.51±0.73 ng/mL) and non-diabetic females (8.15±1.01 vs. 5.97±0.57 ng/ml) was significantly higher than in males of both groups ($p<0.001$). As expected, average HOMA insulin sensitivity was highest in non-diabetic subjects and lowest in diabetic ones ($p=0.004$, age and BMI adjusted data, (Table 1). In addition, HOMA calculations showed that type 2 diabetic subjects had a significant increase in insulin resistance ($p=0.002$). HOMA-β cell function was markedly lower in the poorly controlled diabetic subgroup (88.12±80.90 vs. 125.61±99.26), suggesting that this subgroup had more severe deterioration of their beta cell function than the other subgroup (Table 1). HOMA-β was also markedly lower in males compared to females in the well and poorly controlled subgroups (109.95±87.35 vs. 142.57±112.10 and 59.74±47.04 vs. 130.69±109.17, respectively). The duration of diabetes was 1 to 5 years (mean, 2.80±2.34 yr). Regarding medication usage for control of glucose, 10 patients used metformin, 5 patients used glibenclamide, and 10 used both drugs.

Table 1. Anthropometric characteristics and metabolic parameters of the diabetic and non-diabetic groups

Variables	Diabetics	Non-diabetic controls	p value
Age (years)	44.6±6.3	43.1±9.1	NS
Weight (kg)	92.9±13.63.3	95.5±15.1	NS
Height (cm)	164.6±8.9	163.7±10.8	NS
BMI (kg/m^2)	34.2±3.9	35.5±4.07	NS
Waist (cm)	106.6±10.0	109.4±11.3	NS
Hip (cm)	113.42±10.0	117.2±6.5	NS
Waist to hip ratio	0.93±0.07	0.92±0.08	NS
SBP(mmHg)	130.8±15.1	124.5±13.9	NS
DBP(mmHg)	86.2±10.3	79.1±11.2	0.007
Fasting plasma glucose(mg/dL)	159.6±68.0	90.9±11.8	0.001
Total cholesterol (mg/dL)	180.4±47.7	205.4±55.1	0.047
Triglycerides (mg/dL)	192.6±55.4	168.2±36.1	NS
LDL cholesterol (mg/dL)	108.2±45.6	133.6±51.4	0.032
HDL cholesterol (mg/dL)	34.3±5.7	38.3±7.6	0.016
HbA1c (%)	7.3±2.3	5.1±0.6	0.001
Resistin (ng/mL)	6.4±3.6	7.1±3.7	NS
Insulin (μIU/mL)	19.7±11.4	18.5±10.8	NS
HOMA-IR	7.7±5.8	4.2±2.8	0.002
QUICKI	0.29±0.02	0.31±0.0	0.004
HOMA-B (%)	114.9±94.7	254.5±128.7	0.001

Data are means±SD; $p \leq 0.05$ is considered significant compare within group. BMI, body mass index; SBP,sytolic blood pressure; DBP, diastolic blood pressure; mmHg, millimeters of mercury; LDL, Low- density lipoprotein; HDL, high-density lipoprotein; HbA1c, Hemoglobin A1c; HOMA-IR, Homeostatasis Model Assessment for insulin resistance; QUICKI, Quantitative insulin sensitivity check index; HOMA-B, Homeostatasis Model Assessment for β-cell function

Table 2. Pearson correlation coefficients of resistin with anthropometric and metabolic parameters in diabetic and non-diabetic group

Variables	Diabetic		Non-Diabetic	
	r	p	r	p
Age (years)	0.04	0.80	0.08	0.63
Weight (kg)	0.17	0.31	-0.01	0.93
Height (cm)	-0.11	0.49	-0.19	0.26
BMI (kg/m^2)	0.31	0.06	0.21	0.2
Hip (cm)	0.31	0.06	0.27	0.11
Waist/hip (ratio)	-0.11	0.51	-0.06	0.71
SBP (mmHg)	-0.27	0.11	-0.18	0.28
DBP (mmHg)	-0.30	0.07	-0.38	0.02
Fasting glucose (mg/dL)	-0.16	0.34	-0.17	0.31
Total cholesterol (mg/dL)	0.12	0.47	-0.01	0.99
Triglycerides (mg/dL)	-0.26	0.11	-0.16	0.34
LDL- cholesterol (mg/dL)	0.17	0.31	0.01	0.95
HDL- cholesterol (mg/dL)	0.10	0.54	0.07	0.65
HbA1c (%)	-0.19	0.25	0.18	0.27
Fasting insulin (μIU/mL)	0.30	0.07	0.20	0.25
HOMA-IR	0.21	0.16	0.16	0.35
QUICKI	-0.15	0.68	-0.12	0.47
HOMA-B (%)	0.18	0.29	0.74	0.05

Pearson's correlation analysis was used to evaluate the relationship between serum resistin levels with obesity and insulin resistance markers. We found a trend correlation between resistin and BMI in both groups (Fig.1) In diabetic subjects, we found no correlation between serum resistin levels and any markers of obesity or insulin resistance. In addition, there was no association between resistin levels and lipid profiles (Table 2).

In non-diabetic subjects, the only correlation seen was between serum resistin and diastolic blood pressure (r=0.381, p=0.032). When we compared diabetic and non-diabetic groups, based on males and females separately, the following results were found: There was a significant and positive correlation between serum resistin with weight (Fig. 2) (r=0.456, p=0.050), BMI (r=0.473, p=0.041) and hip circumference (r=0.540, p=0.017) in males of diabetic group (data not shown). There was a positive and significant correlation between serum resistin with waist to hip ratio (r=0.509, p=0.044) and HbA1c (r=0.636, p=0.008) only in males of the non-diabetic control group (data not shown). However in females of the non-diabetic group no significant correlation was seen between serum resistin and BMI (Fig. 3).

There was no significant correlation between serum resistin and any variables in females of either group. In the 24 diabetic subjects, mean HbA1c was 6.12±0.72%, whereas for eleven diabetic subjects this was 10.43±2.09%. The resistin level of 11 subjects who had poorly controlled diabetes was 5.03±2.24 ng/mL, while 24 well controlled diabetic subjects had a resistin level of 6.96±4.00 ng/mL (p>0.05). In order to determine the possible clinical factors influencing the levels of resistin, we performed bivariate regression analyses of resistin and several anthropometric and metabolic factors using the combined data of the non-diabetic and diabetic groups. Although, dBP (β= -0.375, p=0.001), insulin(β=0.295, p=0.007), hip (β= 0.280, p=0.01)and age (β=0.216, p=0.049) were significant independent determinants of serum resistin levels, there was no relationship between resistin and other metabolic or anthropometric markers of insulin resistance and obesity.

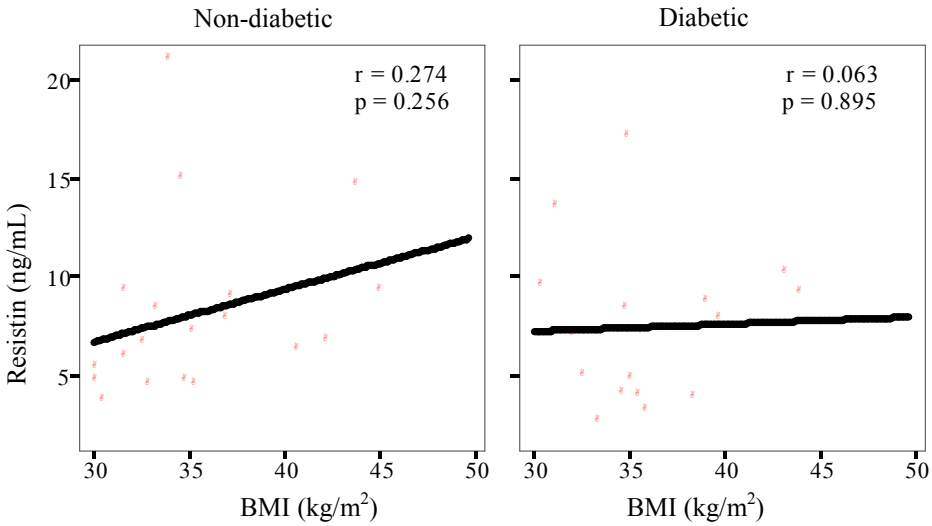

Fig. 1. Relationship between Resistin and BMI in non-diabetic and diabetic groups

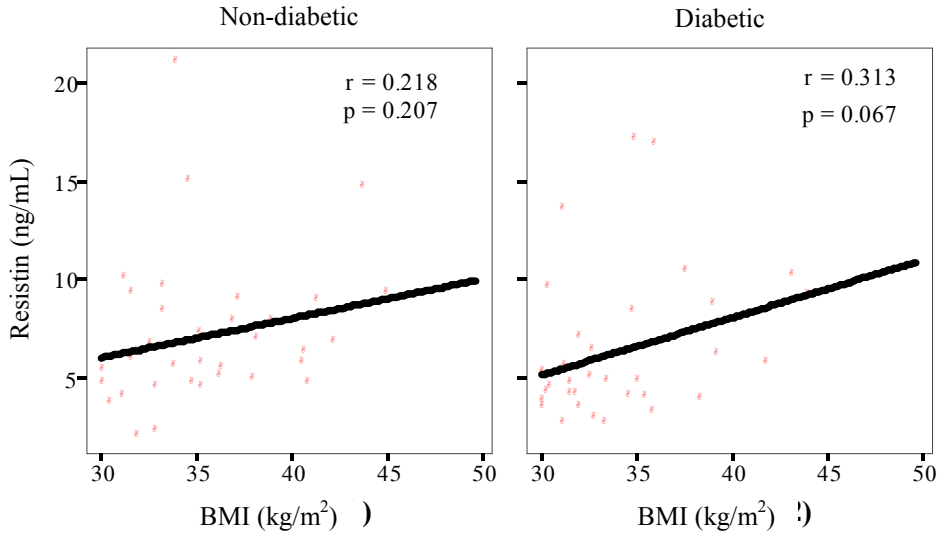

Fig. 2. Relationship between Resistin and BMI in males of non-diabetic and diabetic groups

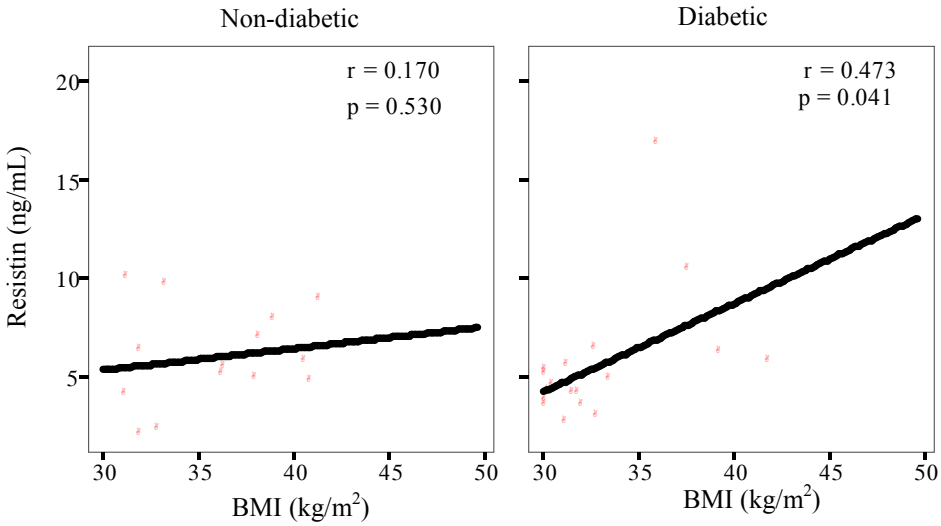

Fig. 3. Relationship between Resistin and BMI in females of non-diabetic and diabetic groups

Discussion

Resistin is an adipocyte secreted hormone that might play a role in obesity, diabetes and insulin resistance. however, its role in humans is largely unclear. In the present study, we demonstrated that serum resistin concentrations did not differ significantly between diabetic (6.40 ±0.61) and non-diabetic (7.16 ±0.62) subjects, confirming results of other studies.[18-21,30] In addition, the present study clearly demonstrated that serum resistin levels did not correlate with any marker of insulin resistance, obesity and anthropometric indices, supporting results of recent other studies.[19-24] Furthermore, we found no significant correlation between resistin and fasting blood glucose, triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol and HbA1c,

though there was a significant difference in serum resistin concentrations between males and females. Although we found no significant correlation between serum resistin and BMI in the two groups, we did find a positive and significant correlation between serum resistin and BMI in the males of diabetic group. The reason for the increase or decrease 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 factors associated with diabetes mellitus, other than insulin resistance, serum glucose level or obesity.

It has been proposed that resistin, secreted by adipocytes may be a factor that links insulin resistance and type 2 diabetes.[37] This proposal is based mainly on the results of in vivo studies in rodent models and investigation of the murine 3T3-L1 adipose cell line. Interpretation of these studies however, has been complicated by contradictory findings on the expression and regulation of resistin.[6,37] In contrast to rodent models, studies in humans have found low levels of resistin mRNA expression1[5-17] and have 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 humans and murine resistin have only 59% homology at the amino acid level9 and that in humans the predominant site of resistin expression is monocytes with low expression in adipocytes.[15,16] Furthermore, in humans, resistin lies on chromosome 19p13.3, a region that has not been linked with susceptibility to obesity or insulin resistance.[37]

Over the past few years, several studies in human have examined the relationship between circulating resistin levels and obesity or diabetes.[18-24,39] 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.[20-22] In contrast, Silha et al.[18] 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 as compared with non-diabetic subjects, but this increase was not associated with markers of insulin resistance or adiposity,[19,23,24] while other studies reported that the serum resistin concentrations were not significant different between diabetic and non-diabetic obese subjects.[25,30-33] Whereas, the results of our study are in general agreement with the latter 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[24] and C-reactive protein[23] were independent determinants of resistin levels. We also showed that the insulin, hip circumference, diastolic blood pressure and age were independent determinants of resistin levels in the combined data of the non-diabetic and diabetic groups.

Furthermore, studies of resistin mRNA and protein expression in adipose tissue of humans have provided additional support that resistin may not play a role in insulin resistance or obesity in humans. Resistin mRNA and protein expression are increased in abdominal fat depots[40,41] and whole adipose tissue of obese individuals, but isolated human adipocytes have very low resistin mRNA levels.[42] In addition, previous studies have reported no differences in resistin expression in human fat and muscle cells when healthy, insulin resistant, and T2DM subjects were compared,[43] and no correlation between resistin gene expression in human adipocytes and insulin resistance[44] or BMI.[42] Finally, studies on the resistin gene and obesity or insulin resistance have revealed inconsistent

results.[45-47] Thus, human studies have not provided consistent evidence that tissue resistin expression plays a key role in the development of insulin resistance.[48]

Although our data do not support the theory that circulating resistin plays a crucial role in insulin resistance and/or obesity in humans, it remains possible that resistin acting in a paracrine or autocrine manner may play a relatively more important role. Future research on the biology of circulating resistin in humans and the role of adipose tissue expression of resistin and RELMs in adipogenesis, obesity, and insulin resistance would be effective in elucidating resistin physiology in humans. Interventional studies including recombinant human resistin administration in the context of well-designed clinical trials or the identification and detailed study of humans with congenital resistin deficiency would definitely be invaluable.

In conclusion, this study demonstrated that the serum resistin concentration differed between diabetic and non-diabetic obese subjects, but this difference was not significant. No significant correlation was observed between serum resistin concentration and insulin resistance or obesity indices. In addition, as calculated by the HOMA model, diabetic subjects had a marked insulin resistance and impaired insulin secretion, which suggests a relative insulin deficiency. Therefore further studies are required to determine the biological function, distribution and cellular source of human serum resistin.

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References

1. Smith SR. The endocrinology of obesity. Endocrinol Metab Clin North Am 1996; 25: 921-42.
2. Reaven GM.Banting lecture 1988. Role of insulin resistance in human disease. Diabetes 1988; 37: 1595-607.
3. Lillioja S, Mott DM, Spraul M, Ferraro R, Foley JE, Ravussin E, et al. Insulin resistance and insulin secretory dysfunction as precursors of non-insulin-dependent diabetes mellitus. Prospective studies of Pima Indians. N Engl J Med 1993; 329: 1988-92.
4. Shimomura I, Funahashi T, Takahashi M, Maeda K, Kotani K, Nakamura T, et al. Enhanced expression of PAI-1 in visceral fat: possible contributor to vascular disease in obesity. Nat Med 1996; 2: 800-3.
5. Spiegelman BM, Flier JS. Adipogenesis and obesity: rounding out the big picture. Cell 1996; 87: 377-89.
6. Steppan CM, Balley ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, et al . The hormone resistin links obesity to diabetes. Nature 2001; 409:307-12.
7. Kim KH, Lee K, Moon YS, Sul HS. A cysteine-rich adipose tissue-specific secretory factor inhibits adipocyte differentiation. Biol Chem 2001; 276: 11252-6.
8. Holcomb IN, Kabakoff RC, Chan B, Baker TW, Gurney A, Henzel W, et al. FIZZ, a novel cysteine-rich secreted protein associated with pulmonary Inflammation, defines a new gene family. EMBO J 2000; 19:4046-55.
9. Steppan CM, Brown EJ, Wright CM, Bhat S, Banerjee RR, Dai CY, et al. A family of tissue-specific resistin-like molecules. Proc Natl Acad Sci U S A 2001; 98:502-6.
10. Gerstmayer B, Kusters D, Gebel S, Müller T, Van Miert E, Hofmann K, et al. identification of RELM-γ , a novel resistin-like molecule with a distinct expression pattern. Genomics 2003; 81: 588-95.
11. Steppan CM, Lazar MA. Resistin and obesity-associated insulin resistance. Trends Endocrinol Metab 2002; 13:18-23.
12. Way JM, Gorgun CZ, Tong Q, Uysal KT, Brown KK, Harrington WW, et al . Adipose tissue resistin expression is severely suppressed in obesity and stimulated by peroxisome proliferator-activated receptor gamma agonists. J Biol Chem 2001; 276: 25651-3.
13. Juan CC, Au LC, Fang VS, Kang SF, Ko YH, Kuo SF, et al. Suppressed gene expression of adipocyte resistin in an insulin-resistant rat model probably by elevated free fatty acids. Biochem Biophys Res Commun 2001; 289:1328-33.
14. Milan G, Granzotto M, Scarda A, Calcagno A, Pagano C, Federspil G, et al. Resistin and adiponectin expression in visceral fat of obese rats: effect of weight loss. Obes Res 2002; 10: 1095-

1103.
15. Savage DB, Sewter CP, Klenk ES. Resistin / Fizz3 expression in relation to obesity and peroxisome proliferator-activated receptor-gamma action in humans. Diabetes 2001; 50:2199-202.
16. Nagaev I, Smith U. Insulin resistance and type 2 diabetes are not related to resistin expression in human fat cells or skeletal muscle. Biochem Biophys Res Commun 2001; 285: 561-4.
17. Engeli JJ, Gorzelniak K, Luft FC, Sharma AM. Resistin gene expression in human adipocytes is not related to insulin resistance. Obes Res 2002; 10: 1-5.
18. Silha JV, Krsek M, Skrha JV, Sucharda P, Nyomba BLG, Murphy LJ. Plasma resistin, adiponectin and leptin levels in lean and obese subjects: correlations with insulin resistance. Eur J Endocrinol 2003; 149: 331-5.
19. Zhang JL, Qin YW, Zheng X, Qiu JL, Zou DJ. Serum resistin level in essential hypertension patients with different glucose tolerance. Diabet Med 2003; 20: 828-31.
20. Azuma K, Katsukawa F, Oguchi S, Murata M, Yamazaki H, Shimada A, et al. Correlation between serum resistin level and adiposity in obese individuals. Obes Res 2003; 11: 997-1001.
21. Lee JH, Chan JL, Yiannakouris N, Kontogianni M, Estrada E, Seip R, et al. Circulating resistin levels are not associated with obesity or insulin resistance in human and are not regulated by fasting or leptin administration: cross-sectional and interventional studies in normal, insulin resistant, and diabetic subjects. J Clin Endocrinol Metab 2003; 88: 4848-56.
22. Degawa-Yamauchi M, Bovenkerk JE, Juliar BE, Watson W, Kerr K, Jones R, et al. Serum resistin (FIZZ3) protein is increased in obese humans. J Clin Endocrinol Metab 2003; 88: 5452-5.
23. McTernan PG, Fisher FM, Valsamakis G, Chetty R, Harte A, McTernan CL, et al. Resistin and type 2 diabetes: regulation of resistin expression by insulin and rosiglitazone and the effects of recombinant resistin on lipid and glucose metabolism in human differentiated adipocytes. J Clin Endocrinol Metab 2003; 88: 6098-106.
24. Young BS, Yu KY, Park HJ, Lee NS, Min SS, Youn MY, et al. Plasma resistin concentrations measured by enzyme-linked immunosorbent assay using a newly developed monoclonal antibody are elevated in individuals with type 2 diabetes mellitus. J Clin Endocrinol Metab 2003; 89: 150-6.
25. American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 2008; 31: S55-S60.
26. Matsuda M, and Defronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp, Diabetologia 1999; 28: 412-9.
27. Shen SW, Reaven GM, Farquhar LW. Comparison of impedance to insulin-mediated glucose uptake in normal subjects and in subjects with latent diabetes. J Clin Invest 1970; 49: 2151–2160.
28. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and B-cell function from fasting plasma glucose and insulin concentrations in man, Diabetologia 1985; 28: 412-9.
29. Hrebicek J, Janout V, Malincikova J, Horakova D, Cizek L. Detection of insulin resistance by simple quantitative insulin sensitivity check index QUICKI for epidemiological assessment and prevention. J Clin Endocrinol Metab 2002; 87: 144-7.
30. Yaturu S, Daberry RP, Rains J, Jain S. Resistin and adiponectin levels in subjects with coronary artery disease and type 2 diabetes. Cytokine 2006; 34: 219-23.
31. Fasshauer M, Paschke R. Regulation of adipocytokines and insulin resistance. Diabetologia 2003; 46: 1594-603.
32. Fehmann HC, Heyn J. Plasma resistin levels in patients with type 1 and type 2 diabetes mellitus and in healthy controls. Horm Metab Res 2002; 34: 671-3.
33. Silha JV, Krsek M, Skrha JV, Sucharda P, Nyomba BL, Murphy LJ. Plasma resistin, adiponectin and leptin levels in lean and obese subjects: correlations with insulin resistance. Eur J Endocrinol 2003; 149: 331-5.
34. Heilbronn LK, Rood J, Janderova L, Albu JB, Kelley DE, Ravussin E, et al. Relationship between Serum Resistin Concentrations and Insulin Resistance in Nonobese, Obese, and Obese Diabetic Subjects. J Clin Endocrinol Metab 2004; 89: 1844 -8.
35. McTernan CL, McTernan PG, Harte AL, Levick PL, Barnett AH, Kumar S. Resistin, central obesity, and type 2 diabetes. Lancet 2002; 359: 46-7.
36. McTernan PG, McTernan CL, Chetty R, Jenner K, Fisher FM, Lauer MN, et al. Increased resistin gene and protein expression in human abdominal adipose tissue. J Clin Endocrinol Metab 2002; 87: 2407.
37. Savage DB, Sewter CP, Klenk ES, Segal DG, Vidal-Puig A, Considine RV, et al. Resistin/FIZZ3 expression in relation to obesity and peroxisome proliferator-activated receptor-gamma action in humans. Diabetes 2001; 50: 2199-202.
38. Nagaev I, Smith U. Insulin resistance and type 2 diabetes are not related to resistin expression in human fat cells or skeletal muscle. Biochem Biophys Res Commun 2001 285; 561-4.
39. Janke J, Engeli S, Gorzelniak K, Luft FC, Sharma AM. Resistin gene expression in human adipocytes is not related to insulin resistance. Obes Res 2002; 10: 1-5.

40. Wang H, Chu WS, Hemphill C, Elbein SC. Human resistin gene: molecular scanning and evaluation of association with insulin sensitivity and type 2 diabetes in Caucasians. J Clin Endocrinol Metab 2002; 87: 2520-4.
41. Sentinelli F, Romeo S, Arca M, Filippi E, Leonetti F, Banchieri M, et al. Human resistin gene, obesity and type 2 diabetes: mutation analysis and population study. Diabetes 2002 ; 51: 860-2.
42. Ma X, Warram J, Trischitta V, Doria A . Genetic variants at the resistin locus and risk of type 2 diabetes in Caucasians. J Clin Endocrinol Metab 2002; 87: 4407-10.
43. Ukkola O. Resistin - a mediator of obesity-associated insulin resistance or an innocent bystander? Eur J Endocrinol 2002; 147: 571-4.