Serum resistin, adiposity and insulin resistance in Saudi women with type 2 diabetes mellitus

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BACKGROUND: The role of adipocyte hormones in modulating insulin sensitivity and glucose tolerance are of increasing interest and importance in studies of type 2 diabetes mellitus. Recently a unique signaling molecule, resistin, has been proposed as playing a role in the pathogenesis of obesity-related insulin resistance, but its relevance to human diabetes remains uncertain. Therefore, we assessed the relationship between serum resistin concentrations and insulin resistance in lean, overweight and obese (OW/OB) non-diabetic and diabetic Saudi women.

SUBJECTS AND METHODS: We measured fasting serum resistin levels in 44 diabetic women with a mean body mass index (BMI) of 31.82 ± 4.35 kg/m^2, 21 OW/OB non-diabetic women with a mean BMI 30.71 ± 3.42 kg/m^2 and in 24 lean women with a mean BMI of 23.33 ± 1.24 kg/m^2. Insulin resistance was assessed using the homeostasis model assessment for insulin resistance formula derived from fasting insulin and glucose levels.

RESULTS: The concentrations of fasting serum resistin showed significant differences among the three groups ($P<0.001$). Mean serum resistin concentrations increased from lean (11.59 ± 2.08) to OW/OB non-diabetic (16.29 ± 2.29) to diabetic (19.42 ± 3.60 ng/mL) women. Significantly higher levels of glucose ($P<0.001$) and values for the homeostasis model assessment ratio (HOMA-R) ($P<0.01$) occurred in the diabetic compared to the lean and OW/OB non-diabetic subjects. Furthermore, resistin correlated significantly and positively with hip circumferences (r=0.39, $P=0.039$), weight (r=0.51, $P=0.005$), insulin (r=0.40, $P=0.033$), HOMA-R (r=0.49, $P=0.007$) and glucose (r=0.39, $P=0.038$) in diabetic women. In OW/OB non-diabetic subjects, resistin correlated with insulin (r=0.59, $P=0.015$) and HOMA-R (r=0.616, $P=0.011$). No correlation was observed with glucose, height, hip, waist, weight, and waist-hip ratio (WHR) in the lean and OW/OB non-diabetic groups.

CONCLUSION: Resistin concentrations are elevated in patients with type 2 diabetes and are associated with obesity and insulin resistance. These data indicate that resistin might be involved in the development of diabetes in humans.

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Diabetes mellitus (DM) remains one of the oldest diseases and the most common metabolic disorder in many populations of the world.[1-3] Type 2 diabetes, characterized by target-tissue resistance to insulin, is strongly associated with obesity. Although the association of obesity with type 2 DM has been recognized for decades, it is not yet completely understood how insulin resistance is associated with increased adiposity. White adipose tissue are known to produce a variety of bioactive peptides, collectively termed adipocytokines, which are considered an important link between obesity and

insulin resistance.[4] A number of metabolic effects have been demonstrated for some adipocytokines, such as leptin, tumor necrosis factor-α and adiponectin, making these molecules candidates in a link between obesity and insulin resistance.[5] Resistin is an adipocyte-secreted polypeptide with a molecular weight of 12.5 kDa. It is one of a family of three proteins, known as resistin-like molecules (RELMs), which have a conserved pattern of 11 cysteine residues at the C-terminal end of the structure.[6] The resistin gene of the mouse has been localized on chromosome 8 whereas its human homologue has been mapped to chromosome 19p13.3.[6] In the human, the role of resistin in the pathophysiology of obesity and insulin resistance is controversial. Although some studies have shown a positive correlation with body fat mass,[7-9] and indeed insulin resistance,[5] others have found no relationship with body mass index (BMI), or insulin sensitivity.[10,11] Resistin is expressed in pre-adipocytes in addition to adipocytes, which may contribute to the elevation of resistin content in the adipose tissue of obese humans.[10,12]

To evaluate a possible role of the newly-identified hormone, resistin, in the development of insulin resistance, we examined the association of serum resistin levels, adiposity, and a measure of insulin action in Saudi women, a population with marked obesity and insulin resistance.[13,14,15] The aim of this study was to determine the level of resistin in lean and overweight and obese (OW/OB) non-diabetic and diabetic Saudi women, and to assess the relationship between serum resisin and insulin sensitivity and fat distribution in Saudi women with type 2 diabetes. To our knowledge this study is the first to examine resistin levels and their relationship to physical and metabolic variables in Saudi subjects.

Subjects and Methods

Eighty-nine Saudi female volunteers, consisting of 45 healthy subjects (24 lean and 21 overweight or obese) and 44 type 2 diabetic patients, aged between 45 and 65 years, were included in this study. Physical data for each volunteer, including weight, height, waist and hip circumferences were recorded. Non-diabetic volunteers were judged to be in good health according to their medical history and their fasting blood glucose (<100 mg/dL). Type 2 diabetic patients taking insulin were excluded from this study

Blood glucose was measured using the *One Touch System* (Johnson & Johnson, USA). Serum resistin concentration (ng/mL) was evaluated in duplicate using the resistin enzyme immunoassay (ELISA) kit

(Phoenix Pharmaceuticals, Inc. USA). The measuring range was 0-500 ng/mL with minimum detectable concentration=0.79 ng/mL. Insulin was determined using the DRG insulin ELISA kit (DRG International, Germany). The degree of insulin resistance was measured using the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) as described by Matthews and coworkers. Homeostasis model assessment of insulin resistance (HOMA-IR), a reliable marker for insulin resistance, was calculated as fasting insulin X glucose level / 22.5.[16]

Statistical analysis of the data was performed using the Statistical Package for Social Science (SPSS for Windows, version 10, 1999, SPSS Inc., Chicago, IL) program. All data were expressed as the mean and standard deviation. Comparison of variables between groups was made by one-way analysis of variance (ANOVA). The least significant difference (LSD) test was used to compare the means for pairs of groups. Correlation coefficients (r) for pairs of variables were determined by Pearson's method to test the strength of association between any two variables. The findings from the bivariate correlation analysis were further explored using stepwise multiple linear regression analysis with resistin concentration as the dependent variable. Differences were considered statistically significant at $P<0.05$ and highly significant at $P<0.01$.

Results

Of the 89 volunteers, there were 24 lean non-diabetic, 21 OW/OB non-diabetic, and 44 diabetic Saudi women (Table 1). Ages ranged between 45 and 65 years and BMI ranged between 20-40 kg/m². By definition, overweight and obese subjects had a higher body mass index (BMI 25-29.9 kg/m² for OW and ≥30 kg/m² for OB). Lean subjects, compared to OW/OB non-diabetic subjects, tended to have a significantly lower BMI and weight, and smaller hip and waist measurements and waist-hip ratio (WHR). Diabetic women had significantly higher waist ($P<0.001$) and hip ($P<0.001$) measurements and WHR ($P<0.001$) than the non-diabetic subjects. Fasting glucose and insulin resistance, as assessed using the homeostasis model of insulin resistance ratio (HOMA-R), were similar in lean and OW/OB subjects, but significantly higher ($P<0.001$) in diabetic compared with non-diabetic women.

Fasting serum resistin was highly significantly ($P<0.001$) different among the three groups (Table 1). Mean serum resistin concentrations increased from lean (11.59±2.08 ng/mL) to OW/OB non-diabetic

(16.29±2.29 ng/mL) to diabetic (19.42±3.60 ng/mL) women. Fasting resistin concentrations were not correlated with age in either the lean, OW/OB non-diabetic or diabetic subjects. Resistin concentrations were not correlated with BMI in lean subjects whereas there was a highly significant positive correlation between resistin and BMI in OW/OB non-diabetic (r=0.49, P=0.002) and diabetic women (r=0.63, P=0.0001). Furthermore, resistin correlated significantly and positively with hip circumferences (r=0.39, P=0.039), weight (r=0.51, P=0.005), insulin (r=0.40, P=0.033), HOMA-R (r=0.49, P=0.007) and glucose (r=0.39, P=0.038) in diabetic women. In OW/OB non-diabetic subjects, resistin correlated with insulin (r=0.59, P=0.015) and HOMA-R (r=0.616, P=0.011). No correlation was observed between resistin and either glucose, height, hip, waist, weight, and WHR among the OW/OB non-diabetic and lean groups. In the stepwise multiple linear regression analysis with resistin concentration as the dependent variable, the HOMA-R (β=0.61, P=0.011) in OW/OB non-diabetic individuals and BMI (β=0.57, P=0.001) and HOMA-R (β=0.39, P=0.01) in diabetic women were significant independent predictors for serum resistin levels.

Discussion

We report resistin levels in non-diabetic and diabetic Saudi women for the first time. Unfortunately, there is little information about resistin blood levels in patients with type 2 diabetes. Most studies of human resistin as well as rodent resistin thus far are genetic studies analyzing the association of the

human resistin locus with diabetes or obesity, and expression studies measuring resistin mRNA levels in these states. Our results showed that circulating levels of resistin were significantly different among lean, non-diabetic OW/OB and diabetic women. Diabetic individuals had the highest resistin concentrations and were characterized by higher waist and hip circumferences than the other two groups. This result identified increased central obesity as a predictive factor for increased resistin concentrations in this population. Several recent studies described findings similar to our results, where significantly higher levels of serum resistin were found in healthy OW/OB subjects than in lean volunteers.[12,17,18] More recently, Youn and his team developed an ELISA for human resistin and measured its plasma concentrations in aged subjects (60-75 years, 199 type 2 diabetic patients and 185 control subjects). Their results indicated a significant increase in resistin level in diabetic patients compared with control subjects.[19] In a further study, serum resistin levels were compared between 74 healthy controls and 90 type 2 diabetic patients and similar results were observed.[20] The mean concentration of resistin in sera from patients and healthy subjects were nearly similar to our results (mean resistin in diabetics, 20.8±0.7 ng/mL vs. healthy subjects, 14.9±0.5 ng/mL). Conversely, another study reported no differences in resistin levels between healthy controls and patients with type 2 diabetes.[21] Also, Silha et al. examined plasma levels of resistin in 17 lean subjects (mean BMI, 23 kg/m^2) and 34 obese subjects (mean BMI, 33 kg/m^2) and found no significant difference in resistin levels

Table 1. Comparison of physical and metabolic characteristics of study subjects (Values are mean±SD).

Variables	Lean (n=24)	OW/OB (non-diabetic) (n=21)	Diabetic (n=44)	P value
Age (years)	43.08±8.64	52.62±7.27	54.29±7.63	<0.01[a,b]
Body mass index (kg/m^2)	23.33±1.24	30.71±3.42	31.82±4.35	<0.001[a,b]
Height (cm)	158.56±4.25	155.63±6.26	156.61±6.41	NS
Hip (cm)	95.33±4.18	110.25±9.38	121.00±10.54	<0.001[a,b,c]
Waist (cm)	74.22±2.44	91.25±7.08	114.68±6.94	<0.001[a,b,c]
Weight (kg)	58.67±4.27	74.19±12.92	78.54±14.70	<0.001[a,b]
Waist hip ratio	0.78±0.03	0.83±0.04	0.95±0.05	<0.001[a,b,c]
Glucose (mmol/L)	3.78±0.56	3.75±0.39	8.74±4.04	<0.001[b,c]
HOMA-R (µU/mL·mmol/L)	1.18±0.53	1.76±0.93	5.38±6.63	<0.01[b,c]
Insulin (µU/mL)	6.90±2.60	10.39±5.18	11.96±5.15	<0.01[a,b,c]
Resistin (ng/mL)	11.59±2.08	16.29±2.29	19.42±3.60	<0.001[a,b,c]

a: lean vs. OW/OB, b: lean vs. diabetic, c: OW/OB vs. diabetic (ANOVA followed by LSD test). NS: non-significant.

between the two groups, but resistin was significantly higher in women compared with men.[5] A very recent study checked serum resistin in 38 non-obese subjects (age, 23±4 years; BMI, 25.4±4.3 kg/m²), 12 obese non-diabetic subjects (age, 54±8 years; BMI, 33.0±2.5 kg/m²), and 22 obese subjects with type 2 diabetes (age, 59±7 years; BMI, 34.0±2.4 kg/m²) and found no differences in resistin concentrations among the three groups.[22] These conflicting data may reflect the lack of adjustment for potential confounding factors, i.e. age and body fat distribution.

The present study also contributed further knowledge of the relationship between resistin levels and obesity-related parameters. The data showed no correlation with resistin in lean subjects, a highly significant correlation with BMI in OW/OB subjects and a highly significant correlation with BMI, WHR, weight, and waist and hip circumferences among diabetic women. These results confirm that an elevated level of resistin is due to release of this protein in obese states, which are characterized by greater waist and hip circumferences. Our findings are similar to the positive relationship between resistin and BMI in other studies in healthy volunteers[9,17] and diabetic subjects.[20] In contrast to our results, no correlation has been previously observed between resistin levels and BMI in diabetic patients or healthy volunteers.[5,21,23]

Regarding the relationship between insulin level, insulin resistance and resistin, we showed that in Saudi women, resistin levels among OW/OB and diabetic subjects were significantly and positively correlated with insulin levels and insulin resistance as assessed by HOMA-IR. Thus, our data add to a growing body of evidence indicating that in humans there is a direct relationship between circulating levels of resistin and insulin. Similar to our results, Zhang et al.[23] reported a positive correlation between serum resistin levels and insulin resistance in 71 non-diabetic subjects (33 men and 38 women). In contrast, some studies failed to show any involvement of

resistin in the etiology of insulin resistance either in rodents[24,25] or humans.[11,26] In two other studies, resistin was not associated with insulin resistance in 30 normal weight patients with renal disease[27] or in 18 obese subjects with acromegaly.[28] Conflicting data on the relationship between resistin, insulin level and insulin resistance are not understood.

Another interesting observation is the increase in insulin resistance scores in diabetic subjects compared with lean and OW/OB subjects. In diabetic women, the prevalence of insulin resistance (HOMA-R ≥3.8) was 43%. The key question is whether resistin is a factor that influences insulin resistance. Our data show that diabetic women have insulin resistance and increased serum levels of resistin and glucose. Obese non-diabetic women did not have a high score for insulin resistance. This suggests that resistin may induce insulin resistance when resistin reaches a certain level, giving rise to the high scores of insulin resistance seen in diabetic women. Alternatively, it is also possible that factors other than an increased resistin level contribute to the high insulin resistance scores in diabetic subjects.

In conclusion, we showed that in a group of healthy and diabetic Saudi females, there was a higher concentration of serum resistin in diabetic women than in healthy women. Additionally, in this study resistin was correlated with diabetes-linked risk factors, obesity and insulin resistance. Therefore, the results of this study support the suggested role of resistin in obesity-related insulin resistance. This suggests that measuring resistin levels could help diagnose people at risk for type 2 diabetes. Lowering the levels of the hormone or blocking its action could constitute a new treatment for type 2 diabetes. Therefore, we recommend that further studies be carried out on a large number of volunteers using a more accurate method to assess insulin sensitivity to clarify the role of resistin in diabetes mellitus and its associated parameters, particularly in humans.

References

1. King H, Rewers M. Global estimates for prevalence of diabetes mellitus and impaired glucose tolerance in adults. WHO Ad Hoc Diabetes Reporting Group. *Diabetes Care.* 1993;16(1):157-77.

2. Jorgensen ME, Bjeregaard P, Borch-Johnsen K. Diabetes and impaired glucose tolerance among the inuit population of Greenland. *Diabetes Care.* 2002;25(10):1766-71.

3. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care.* 2004;27(5):1047-53.

4. Pittas A, Joseph N, Greenberg A. Adipocy-

tokines and insulin resistance. *J Clin Endocrinol Metab.* 2004;89(2):447-52.

5. Silha J, Krsek M, Skrha J, Sucharda P, Nyomba B, Murphy L. Plasma resistin, adiponectin and leptin levels in lean and obese subjects: correlations with insulin resistance. *Eur J Endocrinol.* 2003;149(4):331-5.

6. Steppan C, Brown E, Wright C, Bhat S, Banerjee R, Dai C, Enders G, Silberg D, Wen X, Wu G, Lazar M. A family of tissue-specific resistin-like molecules. *Proc Natl Acad Sci USA.* 2001;98:502-506.

7. Steppan C, Bailey S, Bhat S, Brown E, Banerjee R, Wright C, Patel H, Ahima R, Lazar M. The

hormone resistin links obesity to diabetes. *Nature.* 2001;409:307-312.

8. Savage D, Sewter C, Klenk E, Segal D, Vidal-Puig A, Considine R, O'Rahilly S. Resistin / Fizz3 expression in relation to obesity and peroxisome proliferator-activated receptor-gamma action in humans. *Diabetes.* 2001;50(10):2199-2202.

9. Yannakoulia M, Yiannakouris N, Blüher S, Matalas A, Klimis-Zacas D, Mantzoros C. Body fat mass and macronutrient intake in relation to circulating soluble leptin receptor, free leptin index, adiponectin, and resistin concentrations in healthy humans. *J Clin Endocrinol Metab.* 2003;88(4):1730-1736.

10. Janke J, Engeli S, Gorzelniak K, Luft F, Sharma A. Resistin gene expression in human adipocytes is not related to insulin resistance. *Obes Res.* 2002;10(1):1-5.

11. Lee J, Chan J, Yiannakouris N, Kontogianni M, Estrada E, Seip R, Orlova C, Mantzoros C. Circulating resistin levels are not associated with obesity or insulin resistance in humans 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(10):4848-4856.

12. McTernan C, McTernan P, Harte A, Levick P, Barnett A, Kumar S. Resistin, central obesity, and type 2 diabetes. *Lancet.* 2002;359(9300):46-47.

13. Al-Shammari S, Khoja T, Al-Maatouq M, Al-Nuaim L. High prevalence of clinical obesity among Saudi females: a prospective, cross-sectional study in the Riyadh region. *J Trop Med Hyg.* 1994;97:183-188.

14. Al-Nuaim A, Famuyiwa O, Greer W. Hyperlipidemia among Saudi diabetic patients-pattern and clinical characteristics. *Ann Saudi Med.* 1995;15(3):240-243.

15. Al-Nuaim A. Population-Based epidemiological study of the prevalence of overweight and obesity in Saudi Arabia, regional variation. *Ann Saudi Med.* 1997;17(2):195-199.

16. Mathews D, Hosker J, Rudenski A, Naylor B, Treacher D, Turner R. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentration in man. *Diabetologia.* 1985;28:412-419.

17. Azuma K, Katsukawa F, Oguchi S, Murata M, Yamazaki H, Shimada A, Saruta T. Correlation between serum resistin level and adiposity in obese individuals. *Obes Res.* 2003;11(8):997-1001.

18. Degawa-Yamauchi M, Bovenkerk J, Juliar B, Watson W, Kerr K, Jones R, Zhu Q, Considine R. Serum resistin (FIZZ3) protein is increased in obese humans. *J Clin Endocrinol Metab.* 2003;88(11):5452—5455.

19. Youn B, Yu K, Park H, Lee N, Min S, Youn M, Cho Y, Park Y, Kim S, Lee H, Park K. 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.* 2004;89(1):150-156.

20. Fujinami A, Obayashi H, Ohta K, Ichimura T, Nishimura M, Matsui H, Kawahara Y, Yamazaki M, Ogata M, Hasegawa G, Nakamura N, Yoshikawa T, Nakano K, Ohta, M. Enzyme-linked immunosorbent assay for circulating human resistin: resistin concentrations in normal subjects and patients with type 2 diabetes. *Clin Chim Acta.* 2004;339(1-2):57-63.

21. Fehmann H, Heyn, J. Plasma resistin levels in patients with type 1 and type 2 diabetes mellitus and in healthy controls. *Horm Metab Res.* 2002;34(11-12):671-673.

22. Heilbronn L, Rood J, Janderova L, Albu J, Kelley D, Ravussin E, Smith, S. Relationship between Serum Resistin Concentrations and Insulin Resistance in Nonobese, Obese, and Obese Diabetic Subjects. *J Clin Endocrinol Metab.* 2004;89(4)1844-1848.

23. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman J. Positional cloning of the mouse obese gene and its human homologue. *Nature.* 1994;372:425-32.

24. Juan C, Au L, Fang V, Kang S, Ko Y, Kuo S, Hsu Y, Kwok C, Ho L. Suppressed gene expression of adipocyte resistin in an insulin-resistant rat model probably by elevated free fatty acids. *Biochem Biophys Res Commun.* 2001;289(5):1328-33.

25. Levy J, Davenport B, Clore J, Stevens W. Lipid metabolism and resistin gene expression in insulin-resistant Fischer 344 rats. *Am J Physiol Endocrinol Metab.* 2002;282(3):E626-633.

26. Urbanek M, Du Y, Silander K, Collins F, Steppan C, Strauss J, Spielman R, Legro, R. Variation in resistin gene promoter not associated with polycystic ovary syndrome. *Diabetes.* 2003;52:214-217.

27. Kielstein J, Becker B, Graf S, Brabant G, Haller H, Fliser, D. Increased resistin blood levels are not associated with insulin resistance in patients with renal disease. *Am J Kidney Dis.* 2003;42(1):62-66.

28. Silha J, Krsek M, Hana V, Marek J, Jezkova J, Weiss V, Murphy L. Perturbations in adiponectin, leptin and resistin levels in acromegaly: lack of correlation with insulin resistance. *Clin Endocrinol (Oxf).* 2003;58(6):736-42.